THE Journal OF THE AMERICAN LEATHER CHEMISTS ASSOCIATION

March 2020 Vol. CXV, No.3 *JALCA* 115(3), 77–120, 2020

116th Annual Convention

to be held at the
Eaglewood Resort & Spa
1401 Nordic Road
Itasca, IL 60143

June 16–19, 2020

For more information go to:
leatherchemists.org/
annual_convention.asp

Contents

Distributed by
University of CINCINNATI
LIBRARY PUBLISHING SERVICES

An imprint of the University of Cincinnati Press

ISSN: 0002-9726

Communications for Journal Publication

Manuscripts, Technical Notes and Trade News Releases should contact:
Mr. Steven D. Lange, Journal Editor, 1314 50th Street, Suite 103, Lubbock, TX 79412, USA
E-mail: jalcaeditor@gmail.com Mobile phone: (814) 414-5689

Contributors should consult the Journal Publication Policy at:
http://www.leatherchemists.org/journal_publication_policy.asp

PUT LESS INTO THE ENVIRONMENT AND MORE ON THE BOTTOM LINE WITH BUCKMAN.

You may already look to Buckman to protect hides and safeguard quality. But we can also help you protect the environment and your profitability. Our leather solutions include eco-friendly chemistries and enzymatic technologies that work better, speed up processes, and save energy. So you can dramatically reduce your tannery's environmental footprint. It's *Leatherforward Thinking* from Buckman. Advancing leather. Enhancing production.

To learn more, contact your Buckman representative or visit **buckman.com**.

Buckman™
Chemistry, connected.

JOURNAL OF THE
AMERICAN LEATHER CHEMISTS ASSOCIATION

Proceedings, Reports, Notices, and News
of the
AMERICAN LEATHER CHEMISTS ASSOCIATION

THE JOURNAL OF THE AMERICAN LEATHER CHEMISTS ASSOCIATION (USPS #019-334) is published monthly by The American Leather Chemists Association, 1314 50th Street, Suite 103, Lubbock, Texas 79412. Telephone (806)744-1798 Fax (806)744-1785. Single copy price: $8.50 members, $17.00 non-member. Subscriptions: $185 for hard copy plus postage and handling of $60 for domestic subscribers and $70 for foreign subscribers; $185 for ezine only; and $205 for hard copy and ezine plus postage and handling of $60 for domestic subscribers and $70 for foreign subscribers.

Distributed by the Cincinnati Library Publishing Services (CLIPS), an imprint of the University of Cincinnati Press.

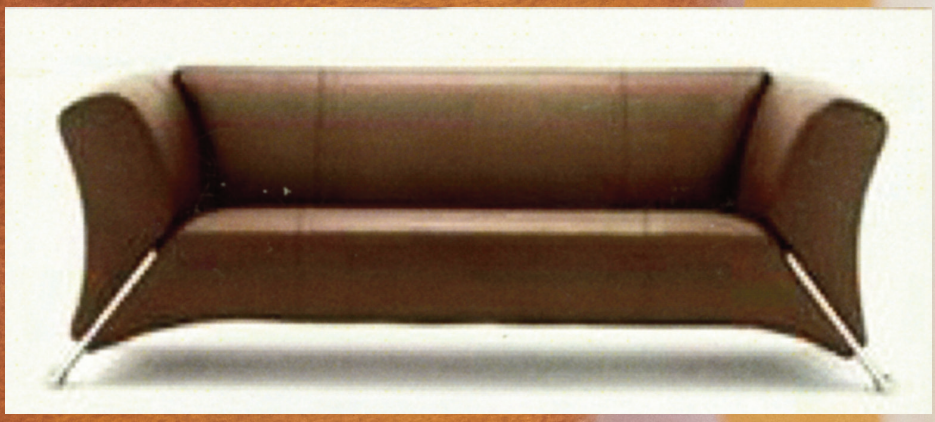
Periodical Postage paid at Lubbock, Texas and additional mailing offices. Postmaster send change of addresses to The American Leather Chemists Association, 1314 50th Street, Suite 103, Lubbock, Texas 79412.

Website: www.leatherchemists.org E-mail: carol.adcock@ttu.edu

LEATHER

AVELLISYNCO

Selected Dyestuffs



CHEMTAN

17 Noble Farm Drive • Lee, NH 03861 (Office)
57 Hampton Road • Exeter, NH 03833 (Manufacturing)
Tel: (603) 772-3741 • Fax: (603) 772-0796
www.CHEMTAN.com

Design of Leather Footwear for Diabetics Containing Chlorhexidine Digluconate Microparticles

by
Fatih Yalcin,[a] Huseyin Ata Karavana,[b]* Seda Rencber,[c] and Sinem Yaprak Karavana[c]
[a]*Technology Transfer Office, Usak University,* Usak, Turkey
[b]*Department of Leather Engineering, Faculty of Engineering, Ege University,* 35100 Bornova-Izmir, Turkey
[c]*Department of Pharmaceutical Technology, Faculty of Pharmacy, Ege University*, 35100 Bornova-Izmir, Turkey

Abstract

ChlorHexidine Digluconate [CHD] was encapsulated inside an ethylcellulose shell material [Aquacoat ECD], and then spray dried to produce mixed microparticles (MPs). The validity and functional quality of the resultant [CHD-MPs] were analyzed on vamp and lining leather which are used to manufacture shoes for diabetics. The morphology, efficiency of encapsulation and *in vitro* release characteristics of the [CHD-MPs] were optimized in order to impregnate [CHD-MPs] onto leather footwear for diabetics. Scanning electron microscopy (SEM) was used to characterize the [CHD-MPs] and the leathers treated with it. SEM images illustrated that the [CHD-MPs] were spherical, smooth in shape and adhered well to leather. *In vitro* CHD-release studies from its MPs, and for leather treated with it were performed in phosphate buffer saline at pH =7.2. There was an inherently controlled release behaviour of CHD for all the formulations on leather. Finally, microbiologic studies on leather treated with [CHD-MPs] were done. This study suggested that footwear containing [CHD-MPs] is/will improve the quality of daily life for diabetics.

Introduction

The number of people with diabetes and foot disease is on the rise. About 15% of the world population [~150 million people] are diabetic. Previous reports highlight that the approximately 15% of people with diabetes worldwide will at some stage develop the health condition of diabetic foot ulceration which could lead to amputation.[1] The estimated prevalence of diabetes for adults between the ages of 20 and 70 worldwide for 2015 was 415 million. Diabetes is expected to affect one person in 10 by 2040 or 642 million people.[2]

Microencapsulation is widely used to provide controlled release of the desired active substance. This technology enables the shell material of the microcapsules to protect the core active substance from adverse reactions, loss or contact with light, heat, and air for a long time with a wall material sensitive to heat, temperature or pH. In addition, impractical core materials can be used effectively with this method.[3,4] In this way, it gives some advantages such as improved efficiency and reduced toxicity.[5] Spray-drying is useful for the microencapsulation technique that has a number of advantages such as continuous particle processing, low-cost encapsulation and the ability to handle labile materials because of the short contact time.[6,7] This technique is most commonly used in many fields such as pharmaceutical, agriculture, biotechnology, cosmetic, carbonless copying paper, footwear, and the food and flavor industry due to low cost and available equipment.[8]

Application of the microencapsulation method in the shoe industry and application of encapsulated core materials to shoe materials will improve the users' welfare and meet their expectations.[9] For example, the fragrance which is applied to the shoe by a microencapsulation method meets one of the basic consumer demands for the removal/masking of bad odors during the use of shoes.[10] In addition, microencapsulation treatments for shoes allow active or ongoing problems to be solved for different purposes: the removal of unwanted odors or the inclusion of antimicrobial agents, increasing the useful life of the microencapsulated shoe or using it in a controlled manner, preventing odors and releasing fragrance to increase the durability of its aroma.[4,11]

Ethylcellulose (EC) is a water-insoluble polymer. It is widely used for microencapsulation of pharmaceuticals to stabilize them against active interactions, hydrolysis and oxidation.[5,12] It has been used particularly as a coating material for sustained release systems.

Aquacoat ECD is an eco-friendly aqueous ethylcellulose dispersion, which matters especially for the preparation of the microparticles (MPs) and coating applications, resulting in environment-friendly technology. It has a solid content of 30% (26% ethylcellulose, 2.4% cetyl alcohol and 1.3% sodium dodecyl sulfate). It requires a plasticizer to decrease the minimum film forming temperature (81°C) and thus improve the film mechanical properties. For most applications, it is recommended that plasticizers are added at a loading of 24% of the latex solids level.[13]

Chlorhexidine digluconate (CHD) was chosen as the model drug in this study because it is an antiseptic agent with topical antibacterial activity. It is a fast-acting biocide that prevents the growth of different bacteria, fungi and yeasts.[14,15] CHD is known to be less toxic to human tissues than other antiseptics, has a high

* Corresponding author E-mail: huseyin.ata.karavana@ege.edu.tr
Manuscript received August 23, 2019, accepted for publication November 21, 2019.

bactericidal activity against a wide spectrum of both gram-positive and gram-negative bacteria and is resistant to inhibition by blood and organic materials.[16,17,18] It is only available as an aqueous solution as the substance cannot be isolated as a solid. CHD is also used as a preservative or disinfectant in a wide range of water-based cosmetics and personal care products, in textiles and in food factories.

The foot activity of diabetics is not adequate; because the veins are clogged and blood circulation has been reduced. This diabetic foot has a reduced sensitivity, is/feels cold and an open wound on it heals slowly because of decreased cell feed.[1,19] If healing is slower than the microorganism growing activity, the condition of the wound may deteriorate which can lead to amputating the foot.[20,21] The suggested footwear for diabetics containing the new [CHD MPs] will be a favorable solution for these patients to improve the quality of daily life. One of the most important problems facing diabetics is microorganism activity; even inside of footwear or an open wound on their feet. Thus, the mere presence of CHD on the footwear surface could reduce the incidence of infection for these patients.

We aimed in this study to produce MPs with Ethyl Cellulose [EC] as a shell material and CHD as active agent therein in order to apply the resultant [CHD-MPs] to vamp and lining leather for the manufacture of shoes for diabetics. Within this scope, MPs loaded with the CHD drug were obtained via co-spray-drying CHD and EC. *In vitro* characterization studies such as loading capacity, encapsulation efficiency and morphological studies of MPs were performed. Additionally, *in vitro* CHD delivery studies of its MPs were performed. Finally, [CHD-MPs] were applied on lining and vamp leather. The presence and efficiency of MPs loaded leather samples were determined in subsequent studies. By the end of the study the controlled release of CHD was enabled successfully on lining and vamp of diabetic footwear leather at the finishing stage of leather making. The controlled release of the CHD in footwear for diabetics will be investigated for comfort and hygiene metrics in a future study.

Experimental

Materials

CHD 20% solution was purchased from Sigma-Aldrich. Aquacoat ethylcellulose dispersion (Aquacoat ECD) was a gift from FMC BioPolymer (Philadelphia, PA). All other materials were of analytical grade. Leathers are prepared via tanned, dyed and all mechanically processed, ready for finish application via spray gun with microcapsule material.

Methods

Preparation of MPs

MPs were carried out in a spray dryer model SD-Basic (Lab-Plant, Huddersfield, U.K). Aqueous ethylcellulose dispersion (Aquacoat ECD) was used as a polymeric system. CHD and Aquacoat ECD were mixed in distilled water. The drug to polymer ratios in the microencapsulating compositions was maintained in 1:1, and 1:2, respectively. The compositions of the formulations in spray-drying are shown in Table I.

Table I

The composition of the formulations

Formulation Code	CHD: Aquacoat ECD
F1	1:1
F2	1:2

The main components of the system are the feed system of the microencapsulating formulation, constituted by a peristaltic pump, a two-fluid atomizer (nozzle diameter of 0.5 mm) and an air compressor; the feed system of the drying gas, constituted by a blower, an air filter, and a temperature control system. The resulting compositions were fed to the spray dryer at the following conditions: pump speed of microencapsulating composition 10 mL/min, inlet air temperature 120˚C and outlet air temperature 80˚C. The dried product was collected during the experiments.

Particle Morphology of the MPs

The MPs morphology via spray dried was determined by a scanning electron microscope (SEM, FEI Quanta 250 FEG). The sample was mounted onto an aluminum stub and sputter-coated with gold-palladium (Au/Pd) using a vacuum evaporator.

Conditions of Ultra-Performance Liquid Chromatography (UPLC)

CHD content of the MPs was determined by UPLC (Thermo Scientific Accela). Separation was achieved on Thermo Hypersil Gold (100 x 2.1mL, 3 µm) C18 column at 25°C by using an isocratic elution method with 0.1% formic acid in water:10 mM buffer solution of sodium phosphate monobasic (pH=3): Acetonitrile (20:40:40) at a flow rate of 300 µL/min This method was validated according to the ICH guidelines.[22]

Encapsulation Efficiency of the MPs

1 mg of the drug-loaded MPs were dissolved in 1 ml of the phosphate buffer saline (PBS, pH 7.4). Then mixed 200 rpm at room temperature for 24 h. In order to determine the amount of active substance loaded in the MPs, the solution was filtered through a 0.22 µm syringe filter and the filtrate analyzed by validated UPLC at 258 nm. The amount of active substance was determined using a calibration curve. Each batch was evaluated five times. The encapsulation efficiency (% EE) was calculated using the following equations:[23]

$$\%\ EE = \frac{B\ (the\ weight\ of\ active\ substance\ found\ in\ MPs)}{A\ (total\ weight\ of\ active\ substance)} \times 100$$

Table II

Basic leather finishing recipe with drug-loaded MPs

Material	Quantity	Application
Water	100 part	
Anionic wax	50 part	
Nonionic aliphatic polyurethane binder	25 part	3x Spray
MPs	12 part	

FT-IR Analysis

FT-IR spectra of the CHD, Aquacoat ECD, and samples were obtained using a Perkin Elmer Spectrum 100 FT-IR spectrometer (Perkin Elmer Spectrum 100, Massachusetts, USA) at room temperature, with wavenumbers ranging from 4000 to 650 cm^{-1}, using four scans with a resolution of 4 cm^{-1}.

In Vitro Drug Release of the MPs

In vitro release studies were performed speed of 100 rpm in phosphate buffer saline (PBS) at 37°C. MPs were suspended in tubes containing 10 mL of PBS. At the appropriate time intervals, the medium in the corresponding tube was filtered through a 0.22 µm filter and the released amount of CHD determined by the validated UPLC method. Sink conditions were maintained in the receptor compartment during *in vitro* release studies. The experiment was carried out five times.

Application of the MPs on the Leathers

MPs were applied on the lining and vamp leathers for diabetic footwear in the finishing process by using spraying pistol. MPs that contained antimicrobial material were added into finishing recipe (Table II) as 20 g per m^2.[24]

Scanning Electron Microscopy (SEM) of the Leathers with the MPs

The morphology of the samples was examined by an SEM (HITACHI TM 1000). The sample was mounted onto an aluminum stub and sputter-coated with gold-palladium (Au/Pd) using a vacuum evaporator.

In Vitro Drug Release of the Leathers with MPs

In vitro release studies were performed speed of 100 rpm in PBS at 37°C. Vamp and lining leathers with 10 cm^2 area were placed in a beaker containing 125 mL of PBS. The samples withdrawn directly at appropriate time intervals were analyzed by a validated UPLC method as previously described. The experiment was carried out five times.

Microbiologic Studies on Leathers with MPs

Agar disc diffusion method was used to examine the effect of drug, MPs, and leather with MPs against the test microorganisms. Test microorganisms were incubated at 37°C for 18 hours in the Muller Hinton Broth (MHB) medium. After incubation, microorganisms were inoculated into Petri dishes containing Muller Hinton Agar (MHA) medium as 10^5 CFU/mL. Then, lining leather samples with 12.7 mm diameter were placed into the Petri dishes. All Petri dishes were incubated at 37°C for 24 hours. Finally, inhibition zones were measured for determining the antibacterial activity.

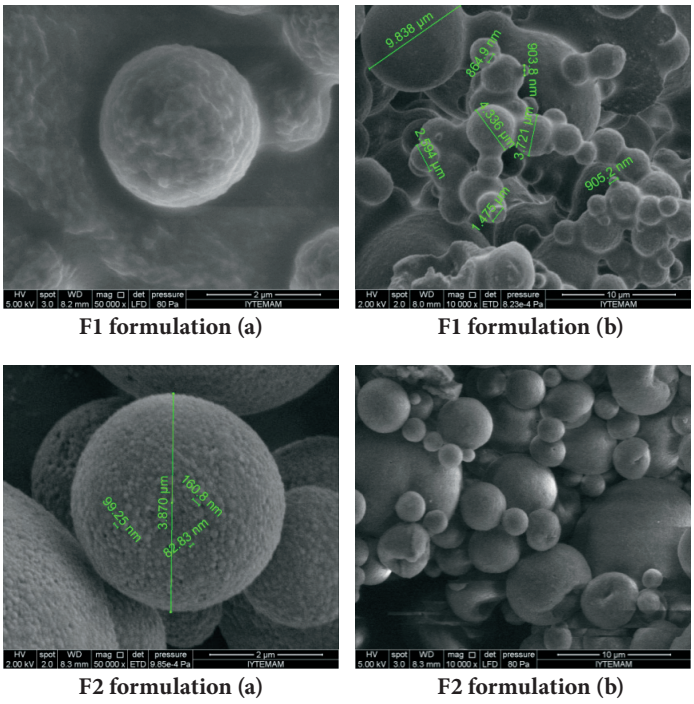


Figure 1. SEM images of MP formulations, F1 formulation at 50000× magnification (a), F1 formulation at 10000× magnification (b), F2 formulation at 50000× magnification (a), F2 formulation at 10000× magnification (b)

Results and Discussion

Particle Morphology

The morphology of the spray-dried MPs was examined by SEM. According to the SEM images, MP formulations had a spherical shape with rough surface morphology. The MPs exhibited irregular shapes also. They do not show the presence of the free drug on their surfaces. These morphological characteristics point out that the CHD is dispersed all over the MPs. Figure 1 showed the morphology of the spray-dried MP formulations. In other spray-drying studies performed by using aqueous polymeric dispersions, MPs with similar morphological characteristics were also obtained.[25,26]

UPLC Conditions

The UPLC method development was carried out according to Havlikova et.al. (2007) method.[22] The method gave a peak of CHD in 1.21 min and the chromatogram is shown in Figure 2. CHD was successfully separated from other compounds. The calibration curve of CHD was linear in the concentration range of 0.5-75 µg/mL (r^2=0.9988). This method was validated according to ICH guideline recommendation Q2 (R1).

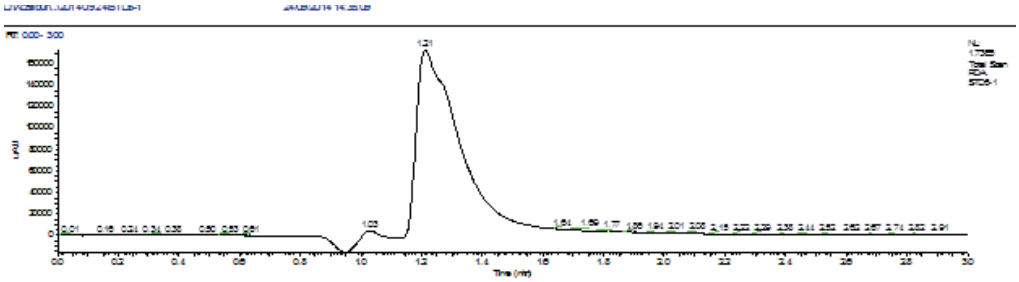


Figure 2. UPLC chromatogram at 258 nm

Encapsulation Efficiency

CHD loaded MPs were produced with a high drug encapsulation efficiency. The encapsulation efficiency of F1 and F2 formulation were found 92.725±5.303% and 88.969±3.250%, respectively. Considering the high encapsulation efficiency, it can be concluded that the spray dryer method is a simple and suitable technique for producing CHD loaded MPs. The results of this study demonstrated that the encapsulation efficiency of the MPs was affected by the drug:polymer ratio. A tendency of a decreased encapsulation efficiency was observed with increasing polymer ratio. This is similar to the effect of the drug:polymer ratio found in the study of Desai and Park (2005).[27]

Fourier Transform Infrared Spectroscopy (FT-IR)

Interaction between the drug and polymer is commonly brought about by identifiable changes in the FT-IR patterns. FT-IR patterns of CHD, Aquacoat ECD and samples are demonstrated in Figure 3-4. Characteristic peaks at 1644, 1530 and 1492 cm^{-1} were exhibited in the FT-IR spectra of CHD (Figure 3-4). The peak 1644 shown also 1643 at leather samples with a new band 1643 (vibration of N-H CHG).[28,29] Also fingerprint of CHG at 1492 peak respectively the aromatic chlorophenol of CHG.[29,30,31] These two bands are shown a bit less quantitatively, according to capsule formulation at 1:2. Moreover, the bands at 1058 (–C–O–C– stretching)[32] and 1376 (due to OH stretching of the intramolecular H-bridge between OH groups)[33]

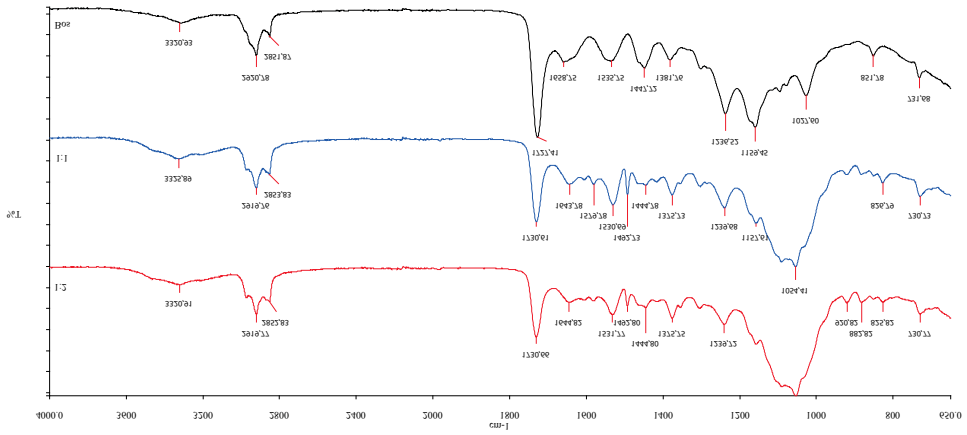


Figure 3. The FT-IR spectrum of the lining leather after without MPs and with two different formulations

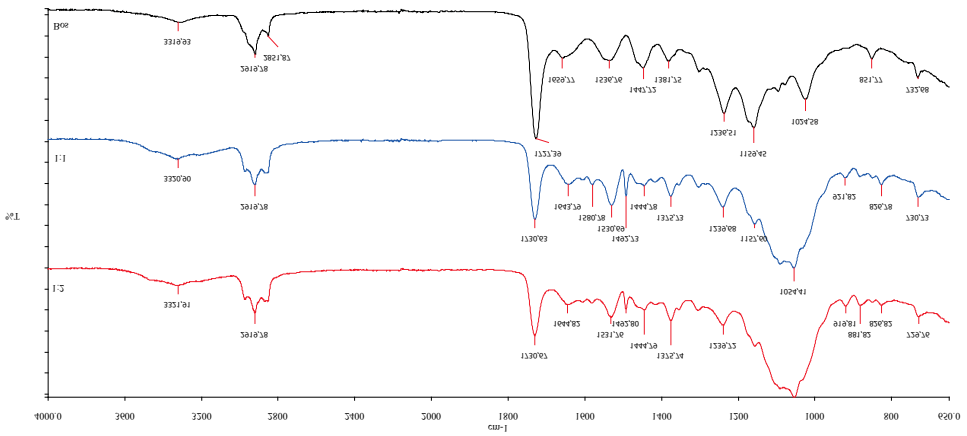


Figure 4. The FT-IR spectrum of the vamp leather after without MPs and with two different formulations

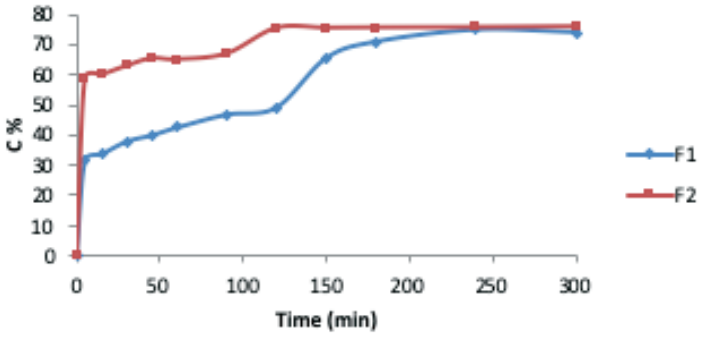


Figure 5. *In vitro* drug release of MPs

bands are characteristic peaks that present at capsules shell material which is ethyl cellulose. By the present band it can be observed that CHG microcapsules have been applied to the leather samples.

In Vitro Drug Release of the MPs

In vitro, drug release studies showed that in CHD release from MPs was very fast. This is probably due to well-done swelling or burst effects. Figure 5 shows *in vitro* release of the MPs. Usually, drug delivery systems are high in the first period due to the application accumulation on the surface of the applied sample.[34] Similarly, MPs also offer initial burst release behavior. MPs prepared by using the spray drying method generally have a matrix structure. Therefore, depending on the method and loading concentration, the drug substance may be present in the MPs as well as on the outer surface when the MPs are exposed to the dissolution media, the drug on the outer surface (non-encapsulated drug) causes a sudden drug release.[35,36]

SEM of the Leathers with the MPs

Electron microscopy images of MP-free and MP-treated with two different formulations vamp and lining leather samples were examined. As seen in Figures 6 & 7, there were more MPs on the F2 formulation samples surface because the F1 formulation's polymer quantity is half of the F2 formulation.

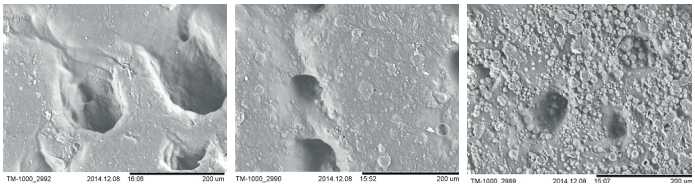


Figure 6. SEM images of the lining leather after finishing process

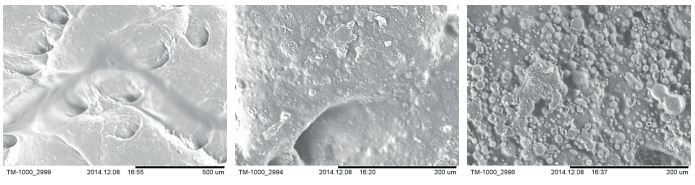


Figure 7. SEM images of the vamp leather after finishing process

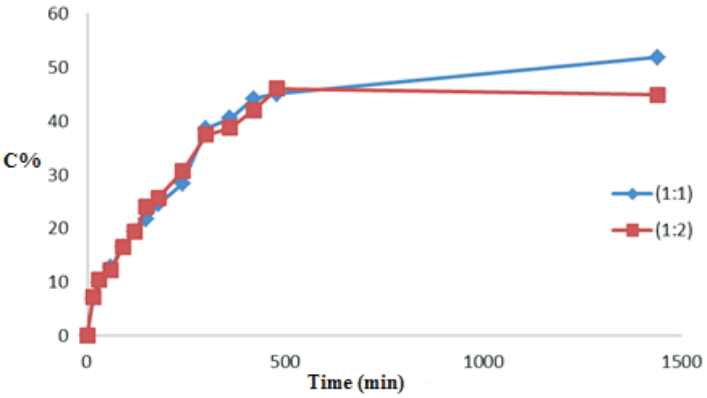


Figure 8. *In vitro* release of the lining leathers with MPs

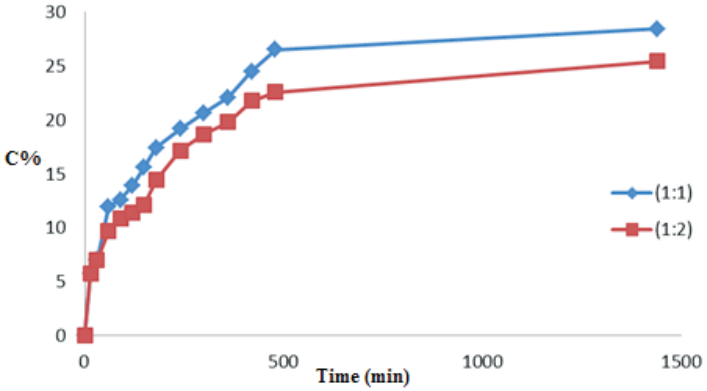


Figure 9. *In vitro* release of the vamp leathers with MPs

In Vitro Drug Release of the Leathers Impregnated with MPs

The *in vitro* release results of the leathers impregnated with MPs in pH 7.4 PBS at 37°C are presented in Figure 8 and Figure 9. As seen in figures, there was controlled release behavior for all formulations from leathers. CHD entrapped within the MPs caused sustained-release more than 24h. Comparing the formulations among each other, the drug release ratio of vamp leather was higher than the release of lining leather up to 72h.

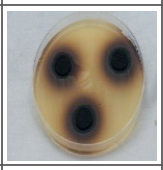
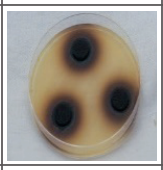
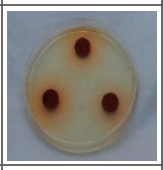
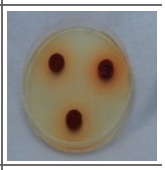
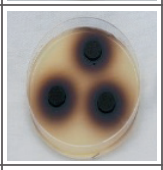
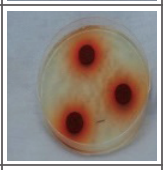
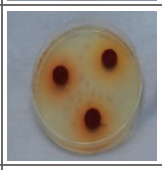
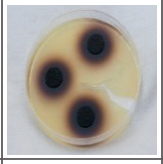
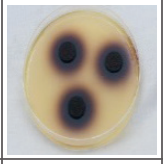
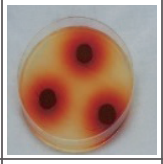
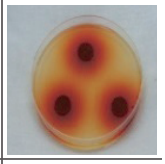
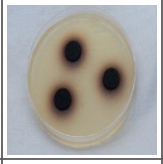
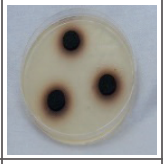
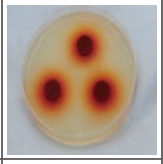
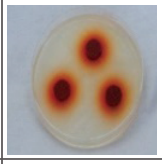
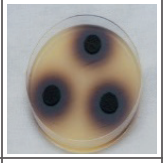
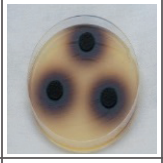
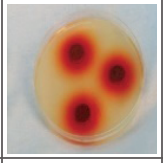
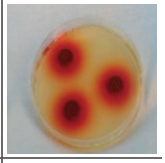
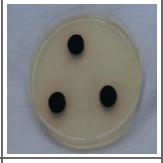
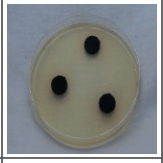
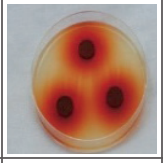
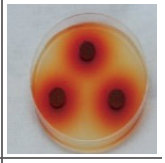
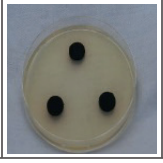
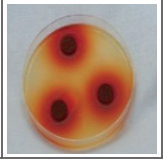
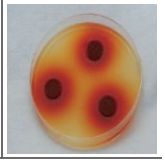
Microbiologic Studies on Leathers with MPs

It was not observed any clear inhibition zone around the vamp and lining leather samples with MPs in Table III. Despite this, on some leather samples, small inhibition zones were seen by microscope. This situation could be interpreted that MPs don't show antimicrobial property comparing the non-capsulated drug. Applying the MPs to the diabetic shoe leathers is affected positively and encapsulation application is used permanence of drug on samples and controlled release.

At all samples, the inhibition zone was not seen, which means that CHD diffusion did not occur. However, the antimicrobial effect can be evaluated with proliferation or without proliferation in the area under the leather samples. This effect is expressed as contact inhibition. It was not seen any proliferation on the contact surface of the vamp and lining leathers in Table III. Also, there

Table III

Microbiologic test results of the leathers with MPs

	Vamp Leather Samples		Lining Leather Samples	
	F1	F2	F1	F2
Staphlylococcus aureus **ATCC 6538-P**				
Escherichia coli **ATCC 12228**				
Pseudomonas aeruginosa **ATCC 27853**				
Candida albicans **ATCC 10239**				
Klebsiella pneumoniae **CCM 2318**				
Enterococcus faecalis **ATCC 29212**				
Staphlylococcus epidermidis **ATCC 12228**				

was not seen any proliferation surface or edge of the vamp and lining leathers.

Conclusion

The active substance [CHD] was dispersed with ethyl cellulose [EC] wall material and then microencapsulated by spray drying. The resultant product [MPs] was applied to vamp and lining leathers to manufacture shoes for diabetics. Aquacoat ECD wall material has been proven to be a useful polymer in the microencapsulation of CHD active substance formulation by spray drying technology in an aqueous system. In this aqueous system, MP production using EC dispersion is an environmentally friendly method because organic solvents are not used. Particle morphology, FT-IR study, encapsulation efficiency and *in vitro* drug release studies on spray-dried MPs were assessed. SEM photographs showed smooth shaped MPs which adhered well to leather and the MPs. The *in vitro* drug release studies showed the release ratio of vamp leather was higher than that for lining leather. CHD drug release was controlled for all formulations onto the leather. The results suggest that leather footwear for diabetics containing CHD-Aquacoat ECD MPs might be a potential medical leather with topical antiseptic properties to support the treatment of diabetics.

Acknowledgement

This work is a part of the authors' research project (No. 113M015) supported by the Scientific and Technological Research Council of Turkey (TUBITAK). The authors acknowledge Ege University Pharmaceutical Sciences Research Center (FABAL) for enabling us to use its laboratory instruments (UPLC).

References

1. Boulton AJM. The diabetic foot: a global view. *Diabetes/Metabolism Research and Reviews* **16**, 1-5, 2000.
2. Facts and Stats, October 2016. Diabetes in the UK 1-3, 2016.
3. Devi N and Kakati DK. Smart porous microparticles based on gelatin/sodium alginate polyelectrolyte complex. *Journal of Food Engineering* **117**:193-204, 2013.
4. Sanchez-Navarro MM, Pérez-Liminana MA, Aran-Ais F, Orgiles-Barcelo C. Scent properties by natural fragrance microencapsulation for footwear applications. *Polymer International* **64**:1458-1464, 2015.
5. Grattard N, Pernin M, Marty B, Roudaut G, Champion D, Le Meste M. Study of release kinetics of small and high molecular weight substances dispersed into spray-dried ethylcellulose microspheres. *Journal of Controlled Release* **84**: 125–135, 2002.
6. Broadhead J, Rouan SKE, Rhodes CT. The Spray Drying of Pharmaceuticals. *Drug Development and Industrial Pharmacy* **18**, 1169–1206, 1992.
7. Benita S. Microencapsulation methods and industrial applications. *Methods of Encapsulation and Advances in Production Technologies* Second Edition, CRC Press, 1-21, *2006*.
8. Gharsallaoui A, Roudaut G, Chambin O, Voilley A, Saurel R. Applications of spray-drying in microencapsulation of food ingredients: An overview. *Food Research International* **40**, 1107–1121, *2007*.
9. Morace F and Ferrarini P. Real Footwear Trends. *Future Concept Lab* Milan, 2012.
10. Sanchez-Navarro MM, Pérez-Liminana MA, Bertazzo M, Aran P, Barcelo CO. Latest Developments in Antimicrobial Functional Materials for Footwear. In: Vilas AV (ed). *Microbial pathogens and strategies for combating them: science, technology and education*. Badajoz: Formatex Research Center, 102-113, *2013*.
11. Sanchez-Navarro MM, Pérez-Liminana MA, Cuesta-Garrote N, Maestre I, et.al. Antimicrobial effect of microencapsulated essential oil mixtures in footwear. In: Vilas AV (ed). *Worldwide Research Efforts in the Fighting Against Microbial Pathogens: From Basis Research to Technological Development,* Brown Walker Press, 102-113, 2012
12. Murtaza G. Ethylcellulose Microparticles: A Review. *Acta Poloniae Pharmaceutica Drug Research* **69**, 11–22, 2012.
13. Aquacoat ECD, http://www.fmcbiopolymer. com/Portals/bio/content/Docs/ AquaCoat%20ECD%207706%20.pdf, (Accessed 23.02.2014).
14. Abad-Villar EM, Etter SF, Thiel MA. Determination of chlorhexidine digluconate and polyhexamethylene biguanide in eye drops by capillary electrophoresis with contactless conductivity detection. *Analytica Chimica Acta* **561**, 133–137, 2006.
15. Labovitiadi O, Lamb AJ, Matthews KH. Lyophilised wafers as vehicles for the topical release of chlorhexidine digluconate—Release kinetics and efficacy against Pseudomonas aeruginosa. *International Journal of Pharmaceutics* **439**, 157–164, 2012.
16. FDA, Classification of Wound Dressings Combined with Drugs. FDA Executive Summary, Advisory Panel, 20-21, September 2016.
17. Sibbald RG, Williamson D, Orsted HL, Campbell K, Keast D, Sibbald D. Preparing the wound bed--debridement, bacterial balance, and moisture balance. *Ostomy Wound Manage* **46**, 14- 22, 2000.
18. Chlorhexidine Digluconate, http://www.ehow.com/about_5675328_chlorhexidinedigluconate_.html, (accessed 27.01.2014).
19. Crawford F, Inkster M, Kleijnen J, Fahey T. Predicting foot ulcers in patients with diabetes: A systematic review and meta-analysis. *QJMed* **100**, 65–86, 2007.
20. Orhan B and Bahçecik N. Technology-Mobile Training in Diabetes and Diabetic Foot Education. *JAREN* **3**, 101-108, 2017.
21. Yuksel A. Diabetes Nursing Basics, In: Prof. Dr. Erdogan S, (ed). *Diabetes Foot Care Diabetes Nursing Association* 127-140, 2002.
22. Havlikova L, Matysova L, Novakova L, Hajkova R, Solich P. HPLC Determination of Chlorhexidine Gluconate and P-Chloroaniline in Topical Ointment. *Journal of Pharmaceutical and Biomedical Analysis* **43**, 1169–1173, 2007.
23. Suwannateep N, Wanichwecharungruang S, Haag SF. Encapsulated Curcumin Results in Prolonged Curcumin Activity *In Vitro* and Radical Scavenging Activity *Ex Vivo* on Skin After UVB-Irradiation. *European Journal of Pharmaceutics and Biopharmaceutics* **82**, 485–490, 2012.
24. Kleban M, Weisser J, Koch F, Schwaiger W. Leather Finished with Scent-Containing Microcapsules. United States Patent Application. US 10/887,685, 2002.
25. Rattes ALR and Oliveria WP. Spray drying conditions and encapsulating composition effects on formation and properties of sodium diclofenac microparticles. *Powder Technology* **171**, 7–14, 2007.
26. Arici M, Topbas O, Karavana SY, Ertan G, Sariisik M, Ozturk C. Preparation of naproxen–ethyl cellulose microparticles by spray-drying technique and their application to textile materials. *Journal of Microencapsulation* **31**, 654–666, 2014.
27. Desai KGH and Park HJ. Preparation and Characterization of Drug-Loaded Chitosan–Tripolyphosphate Microspheres by Spray Drying. *Drug Development Research* **64**, 114–128, 2005.
28. Ricardo N, Gomes de Souza F, Maíra do Prado, Zieberg Passini MZ. Gelatin as a chlorhexidine digluconate immobilizing agent. *MOJ Polymer Science* **1**, 183–186, 2017.
29. Raso EMG, Cortes ME, Teixeira KI, Franco MB, Mohallem NDS, Sinisterra RD. A new controlled release system of chlorhexidine and chlorhexidine:β-cd inclusion compounds based on porous silica. *Journal of Inclusion Phenomena* **67**, 159–168, 2009.
30. Hassiba AJ, E El Zowalaty M, Webstar TJ, Abdullah AM, Nasrallah GK, Khalil KA, Luyt AS, Elzatahry AA. Synthesis, characterization, and antimicrobial properties of novel double-layer nanocomposite

electrospun fibers for wound dressing application. *International Journal of Nanomedicine* **12**, 2205-2213, 2017.

31. Tartari T, Wichnieski C, Bachmann L, Jafelicci Jr M, Silva A, Letra R M, van der Hoeven R, Duarte MAH, Bramante CM. Effect of the combination of several irrigants on dentine surface properties, adsorption of chlorhexidine and adhesion of microorganisms to dentine. *International Endodontic Journal* **51**, 1420–1433, 2018.
32. Gunduz O, Ahmad Z, Stride E. Continuous Generation of Ethyl Cellulose Drug Delivery Nanocarriers from Microbubbles. *Pharmaceutical Research* **30**, 225–237, 2013.
33. Parida P, Mishra N, Sahoo S. FTIR Spectroscopic *In-vitro* Drug Interaction Study of Nifedipine Microsphere. *International Journal of Pharmaceutical Studies and Research* **3**, 1-4, 2012.
34. Kenawy E, Bowlin GL, Mansfield K, Layman J, Simpson DG, Sanders ES, Wnek GE. Release of tetracycline hydrochloride from electrospun poly(ethylene-co-vinyl acetate), poly(lactic acid), and a blend. *Journal of Controlled Release* **81**, 57-64, 2002.
35. Ghorab MM, Zia H. Preparation of controlled release anticancer agents. I: 5-Fluorouracil-ethyl cellulose microspheres. *Journal of Microencapsulation* **7**, 447–454, 1990.
36. Saravanan M, Bhaskar K, Srinivasa Rao GR, Dhanaraju MD. Ibuprofen-loaded ethylcellulose/polystyrene microspheres: an approach to get prolonged drug release with reduced burst effect and low ethylcellulose content. *Journal of Microencapsulation* **20**, 289–302, 2003.

Chromium Leachability from Leather Waste and Eco-toxicity under Environmental Conditions

by

Peng Liang-qiong,[1] Long Wen-jun,[2] Li Yue,[1] Zhang Wen-hua[1,2]* and Shi Bi[1,2]

[1]*The Key Laboratory of Leather Chemistry and Engineering of Ministry of Education, Sichuan University,* Chengdu 610065, Sichuan, China

[2]*National Engineering Laboratory for Clean Technology of Leather Manufacture, Sichuan University,* Chengdu 610065, Sichuan, China

Abstract

Chromium (III) is currently used in leather manufacture worldwide and the attention has been centered on the alleged environmental impact of chromium in leather waste. The effects of liquid-solid ratio, pH, contact time, rotational speed and temperature on the leachability of chromium from leather waste and the eco-toxicity were evaluated to investigate the ecological impact under environmental conditions. The characterization of leachate included the determination of total Cr and total organics (TOC), analysis of UV-visible absorption spectra, as well as the toxicity test with *Photobacterium phosphoreum*. The liquid-solid ratio, extractant pH, contact time and temperature significantly affected the leachability of chromium and toxicity of the leachate, whereas rotational speed slightly affected the leaching behavior when beyond 100 RPM. The toxicity of the leachate was related to both the amount of released substance which was mainly affected by contact time and liquid-solid ratio, and the species which was mainly affected by temperature and extractant pH. Finally, three samples from different tannery corresponding to leather waste were leached and characterized by total Cr, TOC and toxicity test under the environmental conditions of liquid-solid ratio 20:1(mL/g), pH 7.0, contact time 8 hours, rotational speed 100 RPM and temperature 25°C. These results contributed to the risk assessment of stacking field and waste management of chrome-containing leather.

Introduction

It is estimated that about 6.8 million tones leathers are produced worldwide annually, meanwhile about 3.5 million tons of various chemicals are used in leather manufacture.[1, 2] Now about 80% leather is tanned by basic trivalent chromium sulfate, and typical full-chrome tanned leather generally contains 2.0 to 3.0 wt% of chromium.[3, 4] Then solid wastes from tannery, leather product industries, and discarded leather goods may also contain significant quantities of trivalent chromium and other chemicals.[5]0 Currently, in most cases the leather wastes are stacked and disposed through landfill.[6, 7] The chemicals in leather wastes would leach out to overland runoff, soil or even underground water during the leaching of rainwater. So improper stacking and landfill may lead to the release of chromium and other chemicals in leather waste, resulting in potential environmental risk, which often is determined by toxicity and environmental exposure.

The toxicity of leather waste was tested with various organisms such as *photobacterium(P.) phosphoreum*, *daphnia magna* and *sprague-dawley* (rats), and *P. phosphoreum* was found more sensitive to the complex system.[8, 9] Their assay conducted from standard extraction procedure (EP),[10] such as EP test from USEPA,[11] the Spanish legislation, the extraction procedure from the EEC proposal of Directive,[8] and the toxicity characteristic leaching procedure (TCLP) from the USEPA.[12] Indeed, there are a lot of standards or regulations for leaching procedure of solid waste in the world, such as HJT 299-2007 (Sulfuric acid nitric acid method) and HJ 557-2009 (Horizontal oscillation method) in China, and EN 12457-3 of European standard protocol. These procedures are beneficial to reveal the pollution limit and execute strict waste management. Mehmet et al and Yolanda et al investigated the releasing behavior of chromium from leather waste based on TCLP or ISO 17075 standard and a different buffer solution was used as extracting agent.[13, 14] This is different from the situation of stacking yard and landfill site, and the leachable chromium and other chemicals under natural conditions should be further investigated. Furthermore, our previous study showed the adding of organic acids, especially acetic acid, would obviously increase the toxicity of chromium(III) to *P. phosphoreum*.[15] Then the toxicity assay of leather waste should avoid the use of buffer solution when using *P. phosphoreum*.

This study was conducted to investigate the leachability of chromium and other organics from leather waste under natural conditions. Parameters such as liquid/solid ratio, pH, contact time, rotational speed and temperature which affected leaching behavior of chromium and organics in chrome-tanned leather waste were studied. Further the effect of leaching conditions on the toxicity of waste leather was also assessed using *P. phosphoreum*.

*Corresponding author address: zhangwh@scu.edu.cn

Manuscript received October 30, 2019, accepted for publication December 8, 2019.

Materials and Methods

Materials

The chrome-containing finished leather waste with different colors were collected from several tanneries, dried for 48 hours at 45°C and conditioned in standard laboratory atmosphere (20±2°C and 65±5% relative humidity) for 24 hours, then shredded to ≤4 mm using Retsch SM 100 (Retsch GmbH, Germany) mill with rotating knives and a 4 mm sieve and thoroughly homogenized for this study. The samples for the effect of leaching conditions on leachable chromium and toxicity were colored dark blue, and the samples for final test were colored dark blue, red and brown respectively. All chemicals were of analytical grade or better.

Leaching experiments

The leaching experiments were conducted in the thermostatic oscillator (ZWY-2102C, Shanghai, China) with 250-mL conical flask with plug containing leather waste and various volume of water. The effects of liquid-solid ratio (LSR), pH, contact time (CT), rotational speed of oscillator (RS), and temperature (T) on the releasing characteristics of chromium and organics from chrome-containing leather waste under water infiltration were evaluated, respectively. All experiments used ultrapure water (18.2 MΩ/cm, Milli-Q system, Millipore, USA). The extractant pH was adjusted by adding HNO_3 (1 mol/L and 0.1 mol/L) or NaOH (1 mol/L and 0.1 mol/L) to ultrapure water. The experimental details are provided in Table I. After the leaching experiments, the samples were then filtered with 0.45 micron membrane filters and the filtrate was immediately used for analysis and assessment. All the tests were done in triplicate.

Table I

Parameters for the batch leaching experiment

Factor	Variable	Condition
liquid/solid ratio/ (mL/g)	70:1, 60:1, 50:1, 40:1, 30:1, 20:1, 10:1	CT=2h; RS=60 RPM; T=20°C
pH	2.0, 4.0, 6.0, 7.0, 8.0, 9.0, 11.0	LSR=20:1 mL/g; CT=2h; RS=60 RPM; T=20°C
contact time/ h	1, 2, 4, 8, 24	LSR=20:1 mL/g; RS=60 RPM; T=20°C
rotational speed/ (RPM)	30, 60, 100, 150, 200	LSR=20:1 mL/g; CT=2h; T=20°C
temperature/(°C)	20, 25, 30, 35, 40	LSR=20:1 mL/g; CT=2h; RS=60 $r{\cdot}min^{-1}$

Chromium analysis

The samples including leather waste and corresponding leachates were digested to determine total chromium. The leather waste was digested by microwave digestion system(Anton Paar Multiwave PRO, Austria).[16] A 7.0 mL mixed solution of 65% HNO_3 and 30% H_2O_2 (6:1, v/v) was added to 0.10 g sample in a microwave vessel, pre-heated at 130°C for 20 minutes using the matching heating equipment. Then the closed microwave vessel was moved into the microwave digestion instrument. The digestion consisted of a 10-min gradual increase to 1400 W, a 20-min digestion at 1400 W and final cooling to room temperature. The digested sample was diluted with 3% (v/v) HNO_3 and filtered for quantitative analysis of total chromium in leather waste.

The leachates from leaching experiments were digested by the method of wet digestion.[17] 40 mL leachate was placed into 250 mL conical flask, and then 8.0 mL mixed solution of 65% HNO_3 and 30% H_2O_2(3:1,v/v) was added to each vessel. The conical flask was then placed in the electric furnace for digestion. Repeated the process until the solution is clear and transparent. The vessel was washed three to four times with ultrapure water and the rinsed liquor was combined into the same volumetric flask, adjusted to a constant total volume, then mixed thoroughly.

The resulting solutions were analyzed directly by ICP-OES (Perkin Elmer Optima 8000, USA). The calibration standards (1.0-50.0 mg/L) were prepared by diluting the chromium standard stock solutions (1000 mg/L) that obtained from National Nonferrous Metals and Electronic Materials Analysis and Testing Center (Beijing, China). Cr(VI) in leather waste and leachate was also detected by using ISO 17075-1:2017, and National Standard Method of China GB/T 15555.4-1995 respectively.

The leachable chromium of leather waste could be calculated from the concentration of chromium in leachate based on the following equation:

$$A = C \times R \quad (1)$$

Where A represents the mass of leachable chromium from one kilogram of leather waste (mg/kg), C represents chromium concentration in leachate (mg/L), R represents the liquid/solid ratio (mL/g).

Total organics analysis

The released organics in leachate were analyzed by using total organic carbon (TOC) analyzer (DKSH vario TOC, Germany). 5.0 mL leachate was transferred to 25-mL volumetric flask and set to volume by deionized water and the values of TOC and total nitrogen (TN) were obtained directly.

UV-visible absorption spectra

1.0 mL leachate were transferred to 25-mL volumetric flask and set to volume by deionized water for test. UV–visible spectrophotometer (UV-1800PC, Shanghai Mapada Instrument Co. LTD., Shanghai, China) was used to investigate the absorption spectra of the samples with the scanning wavelength range of 190-1100 nm and ultrapure water as a reference.

Toxicological assays

The toxicological assays of leachates were tested by *P. phosphoreum* (T3 mutation, freeze-dried powder supplied by the equipment company) using LumiFox 6000 (Shenzhen Langshi Science Instrument Co. LTD., Shenzhen, China) at 20°C.[18] The *P. phosphoreum* was activated and cultivated to the logarithmic growth stage in the complete liquid culture.[19] The fresh bacteria liquid was diluted using 3% NaCl, till the relative luminous intensity of 50 µL diluted bacteria suspension fell in 50±5% when exposed to 950 µL of 0.10 mg/L $HgCl_2$ standard solution for 15 min. Then the diluted bacteria suspension was used for toxicity assessment. The test was carried out according to the National Standard Method of China (Water quality determination of the acute toxicity – Luminescent bacteria test. GB/T15441 -1995). The pH of samples was adjusted to 6.0 – 8.0 by 0.1 mol/L NaOH and diluted into a series of concentration grades using 3% NaCl. 50 µL of *P. phosphoreum* was added into the tubes containing 950 µL test solution and control (3% NaCl solution), respectively. The relative luminescence intensity was estimated as the following:[20]

$$E\,(\%) = \left[\frac{I}{I_0}\right] \times 100\% \qquad (2)$$

Where E represents the relative luminescence intensity after the 15-min exposure of luminescent bacteria to sample, I is the luminescent intensity of the samples, and I_0 is the luminescent intensity of the blank. EC_{50} is the concentration of sample=corresponding to the relative luminescence intensity of 50% after 15 min of exposure. The lower EC_{50} value indicates higher toxicity.

Results and Discussions

Release behavior of chromium and organics from leather waste

The total chromium in leather waste was determined as 21445.83±1043.10 mg/kg according to the above description which is similar to the previous report.[14] Meanwhile, no Cr(VI) was detected in the waste by ISO 17075-1:2017 method. The release behavior of chromium from leather waste was characterized by the quantitative analysis of total Cr in leachate, and organics in leachate were indicated by both TOC and total nitrogen (TN). As we know, chromium is incorporated into the leather with dominant coordination interaction with collagen and weak adsorption on the collagen matrix.[21] Without damaging the collagen fiber structure under environmental conditions, the leachable chromium should be mainly chromium adsorbed.

Effect of liquid/solid ratio

The liquid/solid ratio is one of the main factors affecting the release of chromium and organics from leather waste. As shown in Fig. 1 the total Cr, TOC and UV-visible absorption of the leachates increased with the decreased liquid/solid ratio. For example, the total Cr, TOC and the absorption of leachate increased by 458% and 144%, and 175% respectively when liquid/solid ratio decreased from 70:1 to 10:1. Furthermore, no peak shift was found in UV-vis absorption spectra with the liquid/solid ratio increasing from 10:1 to 50:1, and 5 nm red-shift was observed when the liquid/solid ratio further increased from 60:1 to 70:1, suggesting the species in leachate varied at high liquid/solid ratio. Based on equation 1, the amount of leachable chromium from leather waste was found to fluctuate around 49.40±0.92 mg per kilogram of leather during the liquid/solid ratio range of 20:1-40:1, indicating such liquid/solid ratio could assess the leachability of chemicals bound weakly.

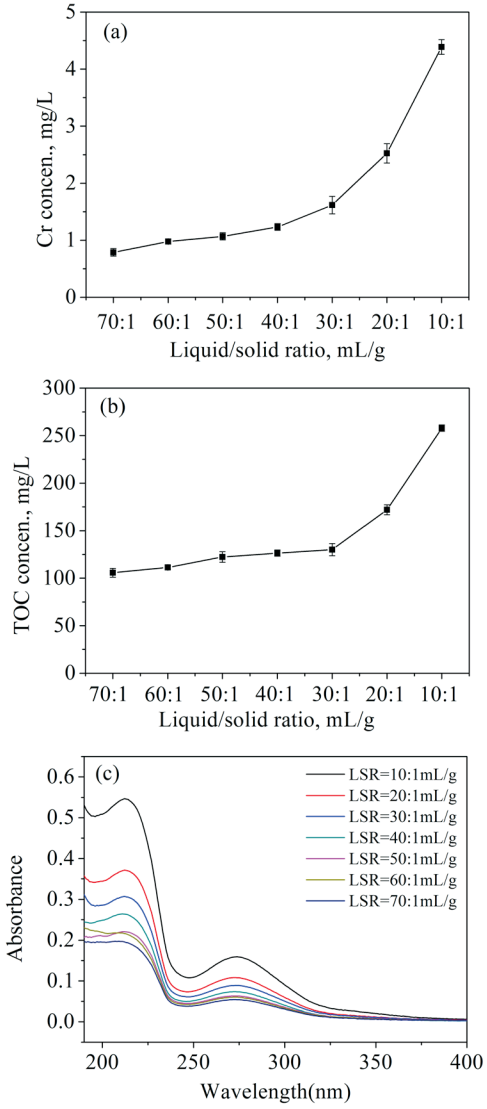


Fig. 1. Effect of liquid/solid ratio on the release of total Cr(a), TOC(b) in leather waste and UV–vis absorption spectra of leachate(c). (Leaching conditions: CT=2h; RS=60 RPM; T=20°C)

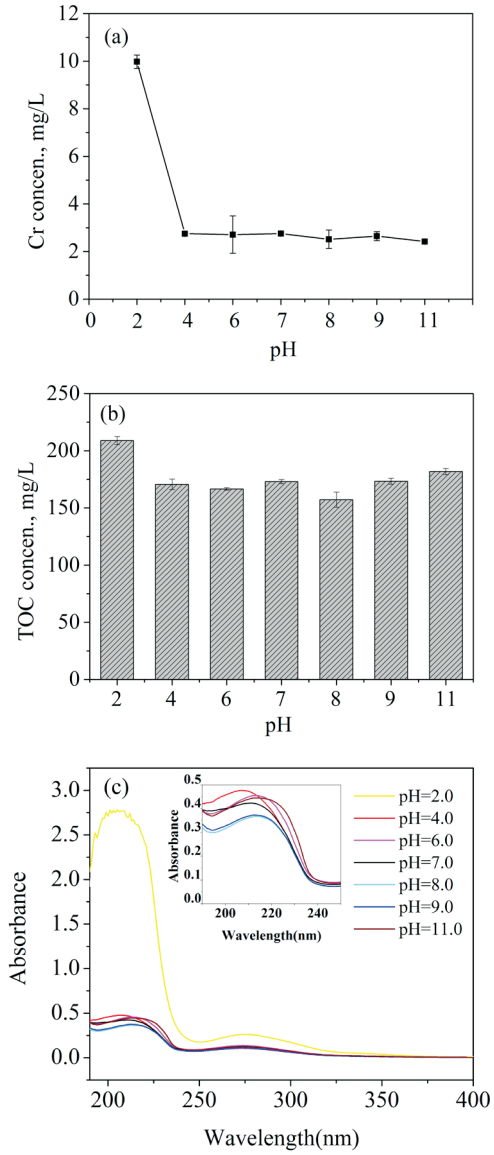


Fig. 2. Effect of pH on the release of total Cr (a), TOC(b) in leather waste and UV–vis absorption spectra of leachate(c).(Leaching conditions: LSR=20:1 mL/g; CT=2h; RS=60 RPM ; T=20°C)

Chromium is the focus of environmental risk assessing on leather waste, however, the chromium concentrations released from leather waste was very low even at L/S ratio of 10:1, ie. 4.39 ±0.13 mg/L, which is below the maximum allowable limit given as 5 mg/L by USEPA and 15 mg/L by national standard of China GB5085.3-2007. This situation means that the waste leather used in this test is not a pollutant. Meanwhile, no Cr(VI) was detected in the leachates by 1,5-diphenylcarbazide spectrometric method.

Effect of pH

The effect of pH on the releasing behavior of Cr and organics was shown in Fig. 2. Except for the leachate with extractant of pH 2.0, the total Cr, TOC and TN of leachate remained around 2.63 mg/L, 170.40 mg/L, and 4.32 mg/L, respectively. The high TN of 104.65 mg/L at pH 2.0 suggested the hydrolysis of collagen fiber, which also led to the release of bonded-Cr and as high as 9.98 mg/L of total Cr in leachate. Then under such extreme acidic conditions, leather waste should be treated as pollutant based on the limit of 5 mg/L by USEPA.

The value of TOC indicated pH slightly affected the release of total organics from leather waste, which decreased from 208.94 mg/L to 170.64 mg/L with the increase of pH from 2. 0 to 4.0, and then remained around 170.40 mg/L with the further increase of pH. From the UV-visible absorption spectra, the increase of pH from 2.0 to 6.0 caused an 8 nm red shift and then the peak position centered at 213 nm if keep increasing pH, indicating the species in leachate was affected by extractant pH especially for acidic extractant. The absorption also indicated the leachable chemicals were slightly higher using deionized water (pH=7.0) as extractant than basic ones.

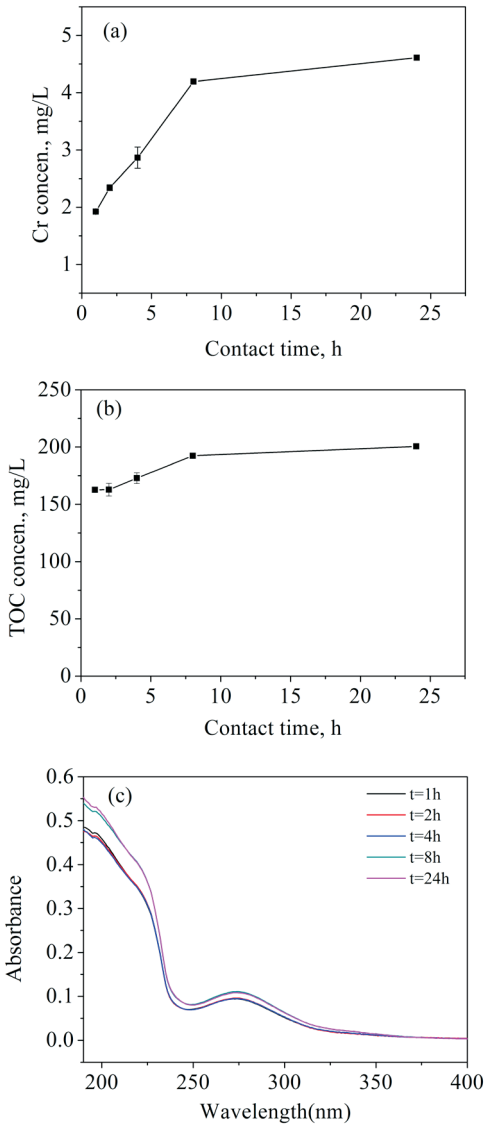


Fig. 3. Effect of contact time on the release of total Cr(a), TOC(b) in leather waste and UV–vis absorption spectra of leachate(c). (Leaching conditions: LSR=20:1 mL/g; RS=60 RPM; T=20°C)

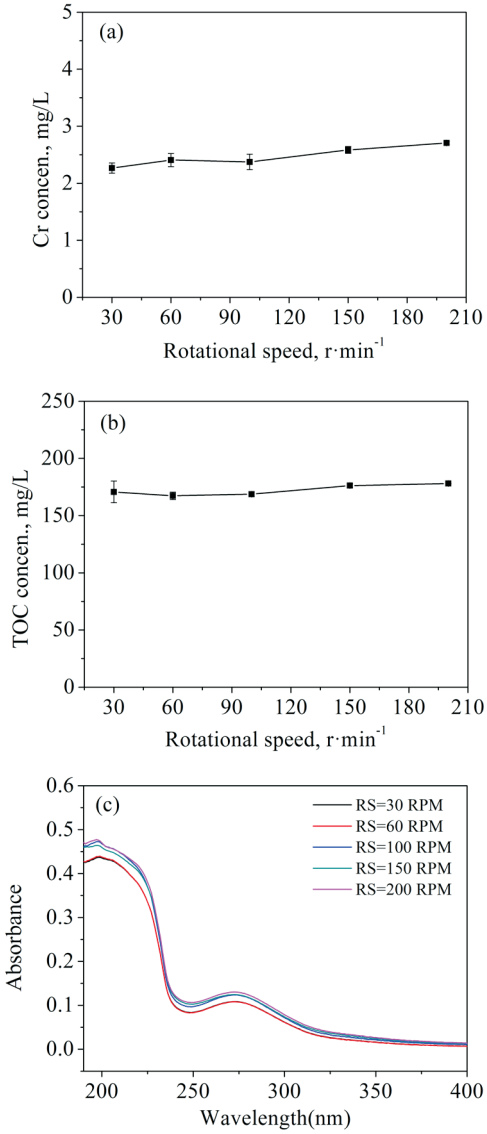


Fig. 4. Effect of rotational speed on the release of total Cr (a), TOC(b) in leather waste and UV–vis absorption spectra of leachate(c). (Leaching conditions: LSR=20:1 mL/g; CT=2h; T=20°C)

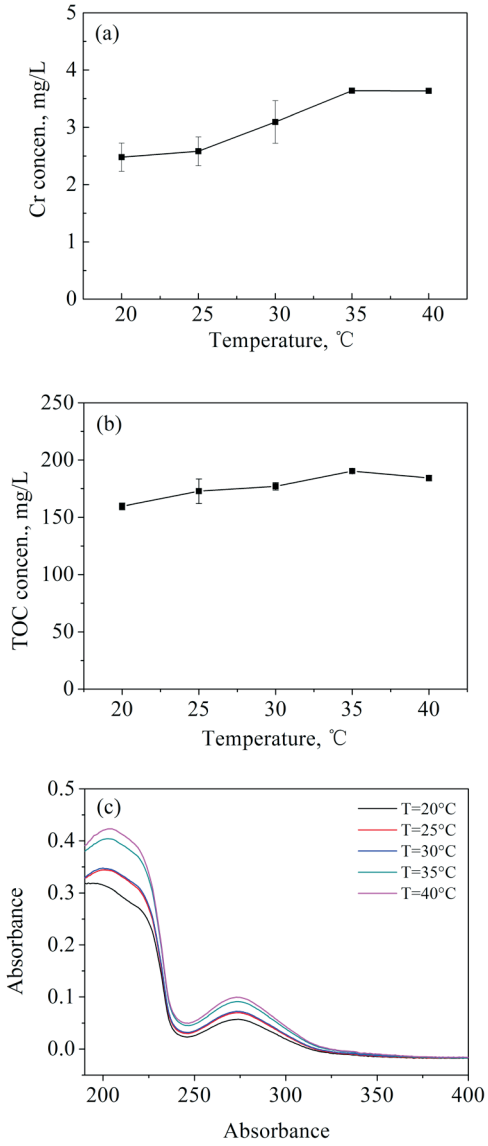


Fig. 5. Effect of temperature on the release of total Cr (a), TOC(b) in leather waste and UV–vis absorption spectra of leachate(c). (Leaching conditions: LSR=20:1 mL/g; CT=2h; RS=60 RPM)

Effect of contact time

Contact time was one of the crucial factors affecting the leaching behavior. As shown in Fig. 3, the total Cr and TOC in leachate increased steadily as the contact time increased from 0 to 24 hours. At the first stage (1- 8 hours), the total Cr and TOC increased rapidly over time. For example, the increase of 118% in total Cr and 18.17% in TOC could be found as the contact time lasted from 1 hour to 8 hours. At the second stage (8 – 24 hours), the total Cr and TOC varied slowly with time, and only increased 9.85% and 4.27%, respectively. From the UV-visible absorption spectra, the increase of contact time caused absorption elevated without the peak shift, indicating only the concentration of leachable substances in the leachate was affected by contact time.

Effect of rotational speed

Effect of rotational speed on chromium and organics leaching from leather waste was shown in Fig. 4. The leachable Cr increased nearly 20% with rotational speed increasing from 30 to 200 RPM. The high rotation caused effective convective transport which would greatly augment external mass transport of chromium leaching,[22] and facilitate the release of chemicals bound weakly. However, the leachable organics increased slowly with rotational speed and TOC value fluctuated during the range of 167.36-178.14 mg/L, indicating minor effect of rotational speed especially above 100 RPM. The result could be further confirmed by the little variance in absorption beyond 100 RPM (Fig. 4c).

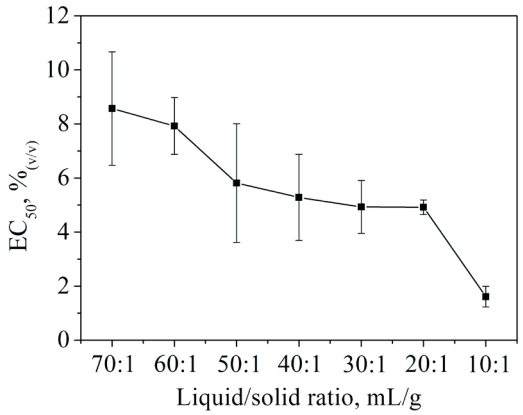


Fig. 6. Effect of liquid/solid ratio on the biotoxicity of the leachate from leather waste. (Leaching conditions: CT=2h; RS=60 RPM; T=20°C)

Effect of temperature

The effect of temperature on the release of chromium and organics from leather waste was investigated at the temperature range of 20 – 40°C. As shown in Fig. 5, the total Cr increased with temperature during the range of 20 – 35°C and then maintained at 3.64 mg/L above 35°C. Meanwhile, TOC of the leachate increased slightly with temperature at the range of 20 – 40°C. From the UV-visible absorption spectra, the increase of temperature caused 11 nm red-shift of maximum wavelength and increased absorbance indicating both species and the concentration of leachable substances in leachate were affected by temperature. However, only 3 nm of red-shift was observed above 25°C. Considering to simulate the leaching of rainwater, a temperature of 25°C is recommended.

Toxicological Assays

Effect of liquid/solid ratio

Investigating the toxic effect of the leachate at different leaching conditions will be helpful for assessing the ecological impact of the chrome tanned leather waste. The effect of liquid/solid ratio on the toxicity of leachate was shown in Fig. 6. The lower EC_{50} value revealed higher toxicity. It could be found that the EC_{50} values of the leachate decreased with the lowered liquid/solid ratio, indicating enhanced toxicity.

The trend of increasing toxicity with lowering liquid/solid ratio may be ascribed to the elevating concentration of chromium and organics in leachates as shown in Fig. 1, which could be further confirmed by the increase of absorbance without shift of peak position especially for the liquid/solid ratio range of 50:1 to 10:1 (Fig. 1c). However, the toxicity of leachate was not linearly related to the concentration of chromium and organics. For example, EC_{50} value fluctuated around 5.24% during the liquid/solid ratio from 50:1 to 20:1, suggesting the liquid/solid ratio in this range could be used to assess the toxicity of leachate.

Effect of pH

The effect of pH on biotoxicity of the leachate from leather waste was shown in Fig. 7. In the range of pH 2.0 to 11.0, the EC_{50} value of the leachates was during volumetric concentration of 3.61% to 8.20%. The toxicity of leachate from acidic extracting agent decreases with the increasing pH, whereas that from basic extracting agent increases with pH and acidic leachate generally showed lower toxicity than basic ones in spite of the same condition in the toxicity assay.

Obviously, pH of extracting agent had a great impact on the toxicity of leachate, which was very different from the release behavior of Cr(III) or organics from leather waste above pH 2.0 (Fig. 2). As we know, the pH of extracting agent would affect the species of chromium and organics especially the organic acid or base, resulting in the difference in toxicity of leachate, which could be confirmed by the red-shift of absorption peak with pH by using UV-visible absorption spectra (Fig. 2c). The basic extracting agent would facilitate the formation of Cr(III)-organic complex, which is generally more toxic to *P. phosphoreum* based on our previous findings.[15] Furthermore, the result suggested the pH of extraction agent should be carefully

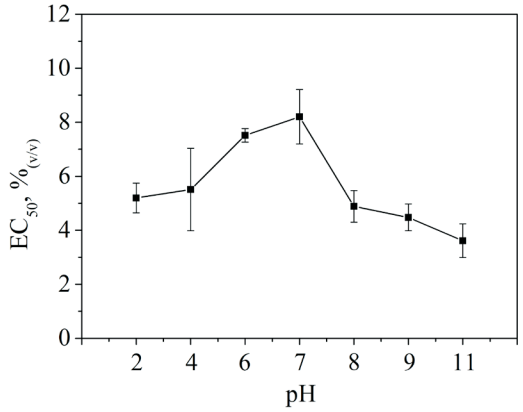


Fig. 7. Effect of pH on the biotoxicity of the leachate from leather waste. (Leaching conditions: LSR=20:1 mL/g; CT=2h; RS=60 RPM; T=20°C)

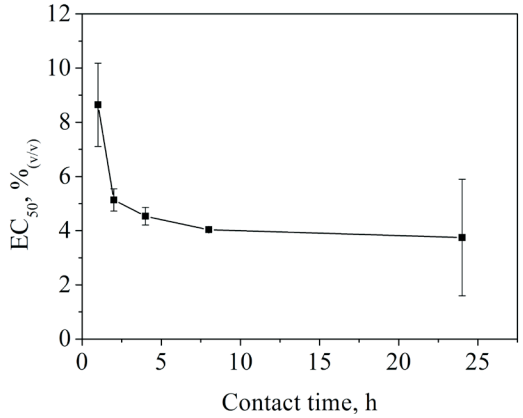


Fig. 8. Effect of contact time on the biotoxicity of the leachate from leather waste. (Leaching conditions: LSR=20:1 mL/g; RS=60 RPM; T=20°C)

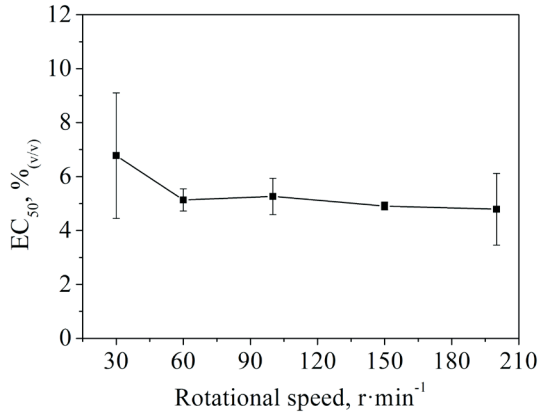


Fig. 9. Effect of rotational speed on the biotoxicity of the leachate from leather waste. (Leaching conditions: LSR=20:1 mL/g; CT=2h; T=20°C)

scrutinized to obtain objective and balanced ecotoxicity assay of leather waste.

Effect of contact time

The effect of contact time on biotoxicity of leachate was shown in Fig. 8. The EC_{50} value of leachate decreased with time, indicating the enhanced toxicity. At the first stage from 1 to 8 hours, the EC_{50} value decreased rapidly from 8.65% to 4.03% over time. At the second stage (8-24 hours), the EC_{50} value varied slightly. The increasing absorption of leachate over contact time without the shift of peak position suggested contact time mainly affected the concentration of leachable substances in the leachate. The change of toxicity with time was in the same tendency as total chromium and organics (Fig. 3), and contact time of 8 hours was enough to assess the toxicity of leachate.

Effect of rotational speed

The effect of rotational speed on the toxicity of leachate from leather waste was shown in Fig. 9. The EC_{50} value was in the range of 4.79% – 6.78%, indicating toxicity slightly increased with rotational speed.

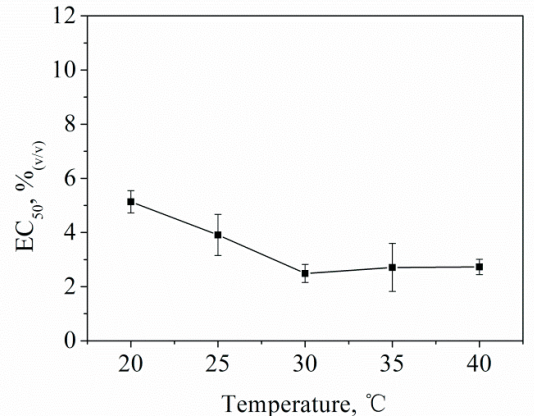


Fig. 10. Effect of temperature on the toxicity of the leachate from leather waste. (Leaching conditions: LSR=20:1 mL/g; CT=2h; RS=60 RPM)

However, the EC_{50} value fluctuated around 5.02% when rotational speed exceeded 100 RPM, which was similar to the varying of Cr and organics with rotational speed as shown in Fig. 4, indicating 100 RPM of rotational speed could assess the toxicity of leather waste.

Effect of temperature

The effect of temperature on the toxicity of leachate from leather waste was shown in Fig. 10. The EC_{50} value decreased with temperature increasing from 20°C to 30°C, and then fluctuated around 2.64% above 30°C. The trend of toxicity with temperature is nearly the same as that of leachable chromium and organics (Fig. 5). From the UV-visible absorption spectra of leachate both the absorption and peak position varied with temperature indicating the amount and species of leachable chemicals were affected by temperature However, only 3 nm of red-shift was observed above 25°C indicating little difference in species.

Obviously, the toxicity of leachate from leather waste depended on the amount of released chromium and organics, as well as the species of released chemicals, which was affected by the leaching conditions. The leaching conditions such as the liquid/solid ratio, contact time and rotational speed mainly affected the amount of released chemicals from leather waste whereas the pH of extracting agent and temperature mainly impacted on the species of released chemicals. Then based on the toxicity assay and leachable total Cr and TOC, the liquid/solid ratio of 20:1, contact time of 8 hours, rotational speed of 100 RPM, temperature of 25°C and deionized water could be chosen to simulate the release of chemicals from leather waste under natural conditions.

Assessment of chrome-tanned leather wastes

Three finished chrome-tanned leather shavings colored dark blue, red and brown were used to assess the leachability and biotoxicity of the hazardous substance under artificial conditions. The total chromium in the three types of leather waste was determined as 23520.57±115.27mg/kg, 30410.70±544.73 mg/kg and 19437.44±465.50mg/kg, respectively, showing the variance of

Table II

Characterization of the leachates of chrome-tanned leather wastes

Item	Cr(mg/L)	TOC(mg/L)	TN(mg/L)	EC_{50}(%)
Sample 1(dark blue)	5.60	253.10	12.79	1.66
Sample 2(red)	3.56	135.16	16.02	1.84
Sample 3(brown)	2.31	129.02	13.40	17.62

chromium in leather waste. The assay was conducted at 25°C under the following leaching conditions: deionized water as the extraction agent, the liquid/solid ratio of 20:1 mL/g, rotational speed of 100 RPM, contact time of 8 hours. These conditions are favorable to the leaching of chromium and organics from leather waste and the results are shown in Table II.

The total Cr in leachate from leather waste colored dark blue was highest among the three samples and exceeded the limit of 5 mg/L given by USEPA, suggesting the leachability of chromium was not relevant to the total Cr in leather waste. The EC_{50} value was negatively correlated with the concentration of chromium and TOC, indicating that the toxicity is definitely correlated with chromium and TOC concentration in leachate. However, the previous experiments have shown that such low levels of chromium do not appear to be highly toxic.[23] Therefore, the biotoxicity of the leachate is related to the interaction of chemical substances in the leachate. The results also indicated the *photobacterium phosphoreum* could easily distinguish the biotoxicity of several finished chrome-tanned leather shavings.

Conclusions

This study was conducted to investigate the leachability of chromium from leather waste under artificial conditions and assess the toxicity. The liquid/solid ratio, contact time and temperature were found to significantly influence the release of chromium and organics from the chrome-containing leather waste and biotoxicity of leachate. With reduced liquid-solid ratio, extended contact time and elevated temperature the concentration of chromium and organics in leachate increased and the biotoxicity also increased. Besides, the pH of extracting agent plays a key role in the biotoxicity of leachate and basic extracting agent led to more toxicity. The rotational speed had relatively little effect on the release of chemicals from leather waste. The following leaching conditions were beneficial to assess the release of chromium and organics: deionized water as extraction agent, liquid-solid ratio 20:1 mL/g, rotational speed 100 RPM, 25°C temperature, contact time 8 hours.

There is a certain correlation between the biotoxicity and the concentration of chromium in leachate. However, the biotoxicity of trivalent chromium was very low at the concentration level of leachate and the high biotoxicity of leachate was ascribed to complicated mixture in spite of the low toxicity of individual component. The results are helpful to understand the leaching rule of harmful substances in chrome-tanned finished leather and its eco-toxicity and to enforce proper management of chrome-containing leather waste.

Acknowledgements

This work was supported by the National Key Research and Development Program of China[2018YFC1802201].

References

1. Lofrano, G., Meriç, S., Zengin, G. E., et al. Chemical and biological treatment technologies for leather tannery chemicals and wastewaters: A review. Sci. *Total Environ.*, **461**, 265-281, 2013.
2. Kanagaraj, J., Senthilvelan, T., Panda, R. C., et al. Eco-friendly waste management strategies for greener environment towards sustainable development in leather industry: a comprehensive review. *J. Cleaner Prod.* **89,** 1-17, 2015.
3. Wells, H. C., Sizeland, K. H., Edmonds, R. L., et al. Stabilizing chromium from leather waste in biochar. *Acs Sustain Chem Eng.* **2**, 1864-1870, 2014.
4. Gong, Y., Liu, X., Huang, L., et al. Stabilization of chromium: An alternative to make safe leathers. *J. Hazard. Mater.* **179**, 540-544, 2010.
5. Gomes, C. S., Repke, J-U., Meyer M. Diauxie during biogas production from collagen-based substrates. *Renewable Energy* **141**, 20-27, 2019.
6. Liu B, Li Y, Wang Q, et al. Green fabrication of leather solid waste/thermoplastic polyurethanes composite: Physically de-bundling effect of solid-state shear milling on collagen bundles. *Compos. Sci. Technol.* **181**, 2019.
7. Pati, A., Chaudhary, R., Subramani, S. J. E. S., et al. A review on management of chrome-tanned leather shavings: a holistic paradigm to combat the environmental issues, *Environ. Sci. Pollut. Res.* **21**, 11266-11282, 2014.
8. Fernández-Sempere, J., Barrueso-Martínez, M. L., Font-Montesinos, R., et al. Characterization of tannery wastes comparison of three leachability tests. *J. Hazard. Mater.***54**, 31-45, 1997.
9. Font, R., Gomis, V., Fernandez, J., et al. Physico-chemical characterization and leaching of tannery wastes. *Waste Manage. Res.* **16**, 139-149, 1998.
10. Heisterkamp, I., Gartiser, S., Kalbe, U., et al. Assessment of leachates from reactive fire-retardant coatings by chemical analysis and ecotoxicity testing. *Chemosphere* **226**, 85-93, 2019.
11. Baba, A., Kaya, A. Leaching characteristics of solid wastes from thermal power plants of western Turkey and comparison of toxicity methodologies. *J. Environ. Manage.* **73**, 199-207, 2004.
12. Gang, Y., Won, E-J., Ra, K., et al. Environmental assessment of contaminated marine sediments treated with solidification agents: Directions for improving environmental assessment guidelines. *Mar. Environ. Res.* **139**, 193-200, 2018.
13. Hedberg, Y. S., Liden, C.,Wallinder, I. O., Chromium released from leather - I: exposure conditions that govern the release of chromium(III) and chromium(VI). *Contact Dermatitis* **72**, 206-215, 2015.

14. Erdem, M., Oezverdi, A., Leaching behavior of chromium in chrome shaving generated in tanning process and its stabilization. *J. Hazard. Mater.* **156**, 51-55, 2008.
15. Zhou, W., Long, W., Xu, T., et al. Organic ligands unexpectedly increase the toxicity of chromium(III) for luminescent bacteria, *Environ. Chem. Lett.* 2019.
16. Ahmed, M., Chin, Y. H., Guo, X., et al. Microwave assisted digestion followed by ICP-MS for determination of trace metals in atmospheric and lake ecosystem. *J. Environ. Sci.***55**, 1-10, 2017.
17. Khan, N., Jeong, I. S., Hwang, I. M., et al. Method validation for simultaneous determination of chromium, molybdenum and selenium in infant formulas by ICP-OES and ICP-MS. *Food Chem.* **141**, 3566-3570, 2013.
18. Tositti, L., Brattich, E., Parmeggiani, S., et al. Airborne particulate matter biotoxicity estimated by chemometric analysis on bacterial luminescence data. *Sci. Total Environ.* **640**, 1512-1520, 2018.
19. Zhu, X-W., Liu, S-S., Ge, H-L., et al. Comparison between the short-term and the long-term toxicity of six triazine herbicides on photobacteria Q67. *Water Res.* **43**, 1731-1739, 2009.
20. Xu, D., Song, X., Qi, W., et al. Degradation mechanism, kinetics, and toxicity investigation of 4-bromophenol by electrochemical reduction and oxidation with Pd–Fe/graphene catalytic cathodes. *Chem. Eng. J.* **333**, 477-485, 2018.
21. Brown, E. M., Taylor, M. M. Essential chromium? *JALCA* **98**, 408-414, 2003.
22. Kim, M., Li, L. Y., Grace, J. R., et al. Compositional effects on leaching of stain-guarded (perfluoroalkyl and polyfluoroalkyl substance-treated) carpet in landfill leachate. *Environ. Sci. Technol.* **49**, 6564-6573, 2015.
23. Peng, L., Li, Y., Han, W., et al. Investigation into the hazards of finished Cr-tanned leather as both product and waste. *J. Soc. Leather Technol. Chem.* **103**, 43-48, 2019.

Antibacterial Activities of Lichen Derived Extracts against Different *Bacillus* Species from Soak Liquor Samples

by
Didem Berber
Marmara University, Department of Biology, Faculty of Arts and Sciences, Istanbul, Turkey

Abstract

In the leather industry, some bacterial strains may become resistant to antibacterial agents utilized in the soaking process due to long-term use and/or not using in sufficient doses. Alternative approaches or novel agents need to be investigated to overcome antibacterial resistance of bacteria present in the soaking process. These alternative approaches may be from natural resources such as lichens which are known to have various biological activities such as antibacterial, antifungal etc. For this purpose, soak liquor samples from different tanneries were collected and eight isolates from these samples were identified by cultural and molecular techniques. Also, the antibacterial effects of acetone extracts of *Hypogymnia physodes, Evernia divaricata, Pseudevernia furfuracea* and *Usnea sp.* at different concentrations were tested on these isolates. They were all Gram (+), rod shaped, oxidase (+), catalase (+), protease (+). Six isolates had lipase activities. The isolates were assigned to *Bacillus toyonensis, B. mojavensis, B. subtilis, B. amyloliquefaciens, B. velezensis, B. cereus,* and *B. licheniformis* in molecular analyses. The acetone extracts of *Evernia divaricata* totally killed *B. toyonensis*, *B. mojavensis, B. amyloliquefaciens,* and *B. subtilis* at the concentrations of 240, 120, 60 and 30 µg/ml, respectively. These extracts had also significant antibacterial efficacies on *B. cereus, B. velezensis, B. licheniformis* at the concentration of 240 µg/ml. The acetone extracts of *P. furfuracea* had a great inhibitory effect on the growth of most species (80.24-88.65%) only at the concentration of 240 µg/ml. *H. physodes* acetone extracts totally killed *B. amyloliquefaciens* and had considerably high suppressive effect on the growth of other tested bacteria at the concentrations of 120 and 240 µg/ml. *Usnea sp.* acetone extracts had inhibitory effect on *Bacillus* species (86.6-97.9%) even at the 30 µg/ml concentration. In this respect, lichens may provide an alternative approach for the leather industry to overcome bacterial resistance to the antibacterial agents.

Introduction

Microbial growth and degradation of hides/skins due to bacterial activities throughout curing and tanning processes are major problems leading to significant economic losses in the leather industry.[14] High moisture and protein contents of freshly slaughtered hides/skins provide a favorable environment for growth of bacteria and as a result, the metabolic activities of these bacteria cause decomposition of hide/skin substances.[5] Therefore, salt or brine curing methods are traditionally used for preservation of slaughtered hides/skins in many countries. These salted or brine-cured raw hides are stored in warehouses or tanneries until beamhouse processes. This storage period may differ according to warehouses or tanneries. Unless raw hides/skins are preserved adequately, bacterial activities and defects on leather will be inevitable. Moderately or extremely halophilic archaea or salt tolerant-non halophilic bacteria may colonize on raw hides/skins. These various types of bacteria have proteolytic and lipolytic enzymatic activities which may cause red and yellow discolorations of the flesh side of hides/skins, bad odor, hair slip, pin pricks, degradation of hair follicle, holes in grain surface, loose grain, grain peeling, disruption of collagen fibers and uneven dyeing in leathers.[6-15] Since environmental conditions in tanneries are available for microbial growth, bacterial population will continue to increase gradually in the subsequent leather processing stages. Thus, these harmful bacteria have considerably significant effects on leather quality in all of the processing stages.

In beamhouse operations, salted hides/skins are primarily soaked in soaking stage to remove salt, blood, soluble proteins, dirt and manure etc. The duration of soaking process depends on countries, preservation methods and tanneries. In our previous questionnaire study, we determined that the soaking process was generally carried out for 12-18 hours in most tanneries in Turkey.[16] The high bacterial numbers and various bacterial species with degradative properties present in soaking liquor may affect the course of soaking process and leather quality. The duration of the soaking should not be too long and should not be performed at higher temperatures (above 22°C).[3] Since the generation time of bacteria can range from 0.5 to 6 hours, bacteria in soak liquor may reach a significant number within 12-18 hours.[16] Therefore, researchers emphasized that effective bactericides must be used to reduce bacterial damage on leather because of high bacterial numbers in the soaking liquor.[17,18] Due to long-term use of antibacterial agents and not using them in sufficient doses, some bacterial strains may become resistant to these antibacterial agents and for this reason, antibacterial efficacy of utilized bactericides in soaking process should be tested regularly.[18] Antibacterial-resistant strains may transfer their resistance genes by horizontal gene transfer[19,20,21] As a result, bacterial growth can occur even in the

*Corresponding author: yazi47@hotmail.com
Manuscript received October 31, 2019, accepted for publication December 17, 2019.

presence of antibacterial substances in the soaking process.[22-24] In an environment containing different populations of microorganisms each bacterial strain may react distinctly than others. Some species disappear, some change, or sometimes bacterial growth is prevented or some are not affected from antibacterial agents. In the meantime, some bacteria may become dominant and more harmful.[19, 25]

Nevertheless, alternative approaches or novel agents with low toxicity, biodegradable and biocompatible properties need to be investigated since antimicrobial agents may not always have the expected effect. However, some chemicals used in the leather industry are related to occupational diseases (bronchial asthma, liver and renal disorders etc.) and may cause toxic effect on liver.[26] These alternative approaches may be from natural resources. Plant based formulations and lichen derived extracts from *Pseudevernia furfuracea* (L.) Zopf. have been reported in the leather industry.[27-29] Throughout the ages, lichens have been used for various purposes, in particular as dyes, perfumes and remedies in folk medicines. In the leather industry, lichens such as *Cetraria islandica* and *Lobaria pulmonaria* have been used as tanning agent from ancient centuries.[30] Lichens are natural inhabitants of many ecosystems which are stable, ecologically obligate symbiotic organisms between fungus (the mycobiont) and one or more algae or cyanobacterium (photobiont). It is known that lichens synthesize unique secondary metabolites that cannot be synthesized by higher plants. Approximately 1050 lichen substances (aliphatic, cycloaliphatic, aromatic and terpenic compounds) have been reported.[31-32] These metabolites are becoming increasingly important due to the need for new bioactive compounds.[33] Various effects of these secondary metabolites such as antimicrobial, antifungal etc. have been discovered. The potential antibacterial effects of various lichen extracts against several bacteria species were reported in the literature.[34-37]

Taken into consideration of all this knowledge, it can be suggested that lichen species with potential antibacterial properties may be effective solution for antibacterial-resistant bacterial strains in soaking process when compared to industrially utilized antimicrobial agents. For this purpose, soak liquor samples from different tanneries were collected and eight isolates were isolated from these liquor samples. They were identified by cultural and molecular techniques. Also, the antibacterial effects of acetone extracts of lichen species *Hypogymnia physodes, Evernia divaricata, Pseudevernia furfuraceae* and *Usnea sp.* on these isolates.

Experimental

Bacterial Strains

Soak liquor samples were collected from different tanneries in Leather Organized Tannery Region, Tuzla-İstanbul, Turkey. Then, these samples were immediately placed into sterile sample bags and carried on ice during transportation. 20 ml of soak liquor samples were put in a flask containing 180 ml 0.85 % sterile physiological saline solution and placed in a shaking incubator (Edmund Bühler, Germany) for half an hour at 25°C in 100 rpm. Direct and serial dilutions of bacterial suspensions were spread onto the Nutrient agar plates. After incubation at 37°C for 24 h, different cream-white colonies were randomly selected to obtain pure culture and spread onto Luria Bertani (LB) agar plates several times. Morphologically different eight isolates were numbered as Isolate 1 to Isolate 8.

Gram Staining, Oxidase-Catalase Tests and Selective Media

Gram staining was performed using earlier described procedures.[38] For oxidase test, 1-2 drops of 1% dimethyl-p-phenylenediamine hydrochloride solution is placed on the filter paper. The colonies were spread over the filter paper which absorbed dimethyl-p-phenylenediamine hydrochloride. Dark blue-purple color formation within 10 seconds was evaluated as positive oxidase activity. 3% H_2O_2 solution was dropped on the colonies for catalase activity and the formation of gas bubbles was recorded as positive catalase activity.[38] Eosine methylene blue agar (Acumedia Lab, Neogen), Baird Parker RPF (BP) (Acumedia Lab, Neogen) agar, Cetrimide agar (Acumedia Lab, Neogen), Mannitol Salt Agar (MSA) (Acumedia Lab, Neogen) and 5% sheep blood agar were used to confirm the presence of the strains belonging to family *Enterobacteriaceae*, genera of *Staphylococcus*, *Pseudomonas* and *Bacillus*. All experiments were done duplicate.

Protease and Lipase Activity

Protease activities of eight isolates were tested on gelatin agar medium containing 2% gelatin (w/v). After 24 h incubation, the agar plates were flooded with Frazier solution. Clear zones around the colonies evaluated as positive for the protease activity. Lipase activities of the isolates were examined on agar medium containing tripticase pepton (0.8%) and Rhodamin B (0.2 mg/100 ml). Fluorescent orange halos around the colonies were evaluated as positive lipase activity.[39,40]

Molecular Analyses

Genomic DNA of tested isolates were extracted by phenol/chloroform extraction and ethanol precipitation. DNA isolation was confirmed by agarose gel electrophoresis and ethidium bromide staining. All DNA from sample were stored at –20°C until use. The 16S rRNA gene was amplified by Polymerase Chain Reaction (PCR) with the universal bacterial primers 27F (5-AGAGTTTGATCMTGGCTCAG) and 1492R (5-TACCTTG TTACGACTT).[41] Negative control was included in PCR amplifications. PCR amplification was carried out by initial denaturation at 95°C for 4 min, followed by 30 cycles at 95°C for 1 min, 57°C for 1 min, and 73°C for 1 min. the reactions were finished by a final extension at 73°C for 7 min. The PCR products were monitored by agarose gel electrophoresis. These products were purified by *Thermo Scientific*™ GeneJET™ *Gel Extraction Kit.* These purified samples were analyzed by MedSanTek Laboratory Istanbul (Turkey). The 16S rRNA sequence contigs were generated by the software ChromasPro version 2.1.8 (Technelysium Pty. Ltd, Tewantin, Queensland, Australia) Then, consensus sequences were exported in Fasta format for each sample for data analysis. These sequences compared with sequences in the NCBI database using the BLAST search program.

Lichen Samples

Hypogymnia physodes, Evernia divaricata, Pseudevernia furfuraceae and *Usnea sp.* were collected to be 20-30 gr in quantities from fir trees of Kastamonu province in the north-west of Turkey. They were identified through classical taxonomical methods by microscopic examination. Voucher specimens were deposited with the lichen collection of Marmara University Herbarium (MUFE).

Hypogymnia physodes, Evernia divaricata, Pseudevernia furfuraceae and *Usnea sp.:* Turkey, Kastamonu province, Kapaklı Village, 41.24492, 34.18330, G.Çobanoğlu.

Extraction of Lichen Samples

After lichen samples were washed and dried on air, they were weighed and pulverized by liquid nitrogen in porcelain mortar. The lichen samples were taken into sterile bottles, acetone (grade: ACS,ISO,Reag. Ph Eur) was added and then kept in a dark place for 24 hours. Following the evaporation of acetone in a rotary evaporator, crude lichen acetone extracts with extract yields of approximately 2.5% for the lichen species were obtained.

Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentration (MIC) tests were performed in 96-well microplates (Greiner Bio-One, Cell Star, F-bottom, with lid). Tryptic soy agar was added to each well and four-fold serial dilutions of the acetone extracts of *H. physodes, E. divaricata, P. furfuracea* and *Usnea sp.* were made. Final concentration of lichen extract were 240, 120, 60 and 30 µg/ml. Overnight culture of Isolate A was added to obtain a total volume of 100 µl with an OD_{600} of 0.1. The experiments included positive and negative controls. The tests were performed in three replicates. The bacterial growth was evaluated at 20th hour using Cytation 3 multimode microplate reader (Biotek), by measuring absorbance.

Results and Discussion

In this study, eight isolates, which were obtained from soak liquor samples collected from different tanneries in Istanbul Organized Leather Industrial Zone, Turkey, were identified by cultural and molecular techniques. In addition, for the first time, potential antibacterial properties of acetone extracts of *H. physodes, E. divaricata, P. furfuracea* and *Usnea sp.* were evaluated against these isolates.

Previous studies emphasized that bacterial contamination may occur from the beginning of salt curing method due to improper preservation and the number of these bacteria will possibly increase further throughout the soaking process.[42] It has been known that a variety of microorganisms may be present in soak liquor samples. In the present study, eight isolates, which are morphologically different in Nutrient agar, were detected to be Gram positive, rod shaped, oxidase and catalase positive. Whereas the bacterial growth was observed on Mannitol Salt Agar (MSA) and 5% sheep blood agar, no growth was detected on EMB and Cetrimide agar. These results suggested that these isolates may be belonging to the genera of *Bacillus* due to the characteristics of Gram staining, cell morphology and catalase test. As known, *Bacillus* may grow on MSA since this

Table I

Isolate codes, closest relatives, similarities, Gram staining, oxidase and catalase tests, bacterial growth on selective media, protease and lipase activity results of eight isolates collected from soak liquor samples of different tanneries.

Isolate code	Closest relative	Similarity %	Genbank Accession Number	Gram Staining	Oxidase test	Catalase test	EMB agar	BP Agar	Cetrimide Agar	MSA	5% Sheep blood agar	Protease activity	Lipase activity
1	*Bacillus toyonensis*	100	MN428224	+	+	+	–	+¥	–	+Y	α	+	+
2	*Bacillus mojavensis*	99.93	MN120046	+	+	+	–	-/+¥	–	+Y	α	+	+
3	*Bacillus cereus*	100	MN428211	+	+	+	–	+¥	–	+*	β	+	–
4	*Bacillus velezensis*	99.93	MN240443	+	+	+	–	+¥	–	+Y	α	+	+
5	*Bacillus cereus*	100	MN232161	+	+	+	–	+¥	–	+*	β	+	–
6	*Bacillus licheniformis*	98.08	MN368416	+	+	+	–	+¥	–	+Y	–	+	+
7	*Bacillus amyloliquefaciens*	99.72	CP035899	+	+	+	–	+¥	–	+Y	α	+	+
8	*Bacillus subtilis*	100	MN208471	+	+	+	–	+¥	–	+Y	α	+	+

¥Cream colored colonies *Pink–cream colored colonies YYellow colonies + growth – no growth -/+ weak growth

genus is including salt-tolerant members. These suggestions were confirmed by no bacterial colonization on EMB and Cetrimide agar. All isolates had protease activities whereas six isolates had lipase activities. Experimental data of cultural methods were included in Table I.

In molecular analyses, tested eight isolates were identified by comparative partial 16S rRNA gene sequence analysis with the sequences deposited in the GenBank database via BLAST program. It was determined that these isolates have similarities with *Bacillus toyonensis, Bacillus mojavensis, Bacillus subtilis, Bacillus amyloliquefaciens, Bacillus velezensis, Bacillus cereus,* and *Bacillus licheniformis*. The percentage of similarities and GenBank accession numbers belonging to tested isolates were given in Table I.

Before phylogenetic tree construction by using the neighbor-joining method, multiple-alignment was performed in the ClustalW program. Mega X software was used for phylogenetic tree. The evolutionary distances were showed on phylogenetic tree with branch. GenBank accession no. was indicated at each branch.

Recently, Yılmaz and Birbir (2019) isolated *B. mojavensis, B. licheniformis, B. velezensis, B. amyloliquefaciens, B. subtilis, B. atrophaeus, B. paralicheniformis, B. safensis, B. siamensis, B. tequilensis, B. pumilus,* and *B. halotolerans* from curing salt samples. The researchers indicated that curing salt contaminated with *Bacillus* species may lead to dominancy of bacterial species belonging to this genus on hide/skin samples during different stages of leather processing. Similarly, *B. mojavensis, B. licheniformis, B. velezensis, B. amyloliquefaciens,* and *B. subtilis* were also isolated from soak liquor samples in the present study. These results indicate that these *Bacillus* species may come from curing salt samples and may survive during the soaking process despite the antibacterial agent utilization.

In previous studies, the existence of bacteria belonging to the genus *Bacillus* and species of *B. cereus, B. laterosporus, B. liquefaciens, B. megaterium, B. subtilis,* and *B. pumilus* were reported from soak liquor samples[3, 9, 42, 44-46]. In our previous study evaluating the efficacy of antimicrobial agent (the active content, didesyldimethylammonium chloride), *Bacillus mycoides, Bacillus lentus,* and *Bacillus amyloliquefaciens* were isolated despite the applied doubled concentration of antimicrobial agent in soak liquor samples.[18] Unlike these studies, *Bacillus toyonensis* was isolated and identified in this study. Due to high numbers of proteolytic and lipolytic non-halophilic bacteria and the high content of organic matter in the hide/skin, bactericide utilization in sufficient concentration in soaking process sometimes may not be effective to reduce the number of bacteria. The adverse effect of *Bacillus* species on leather quality was evaluated by Rangarajan et al., (2003) and they observed perforations in grain surface due to *Bacillus subtilis, B. megaterium, B. anthracoides, B. pumilus*. They emphasized that antimicrobial agents should be added regularly especially in summer or when hides/skins are soaked for longer periods.[42] In particular, it is difficult to inactivate proteolytic strains which have penetrated into the hide/skin and attached to collagen fibers via high concentrations of bactericides. Therefore, these bacteria will continue to multiply and damage the collagen fibers of the skin.

The antibacterial effect of lichen samples against *Bacillus* genus from various sources was reported in the literature. The acetone and chloroform extracts of *P. furfuracea* (L.) Zopf was also tested

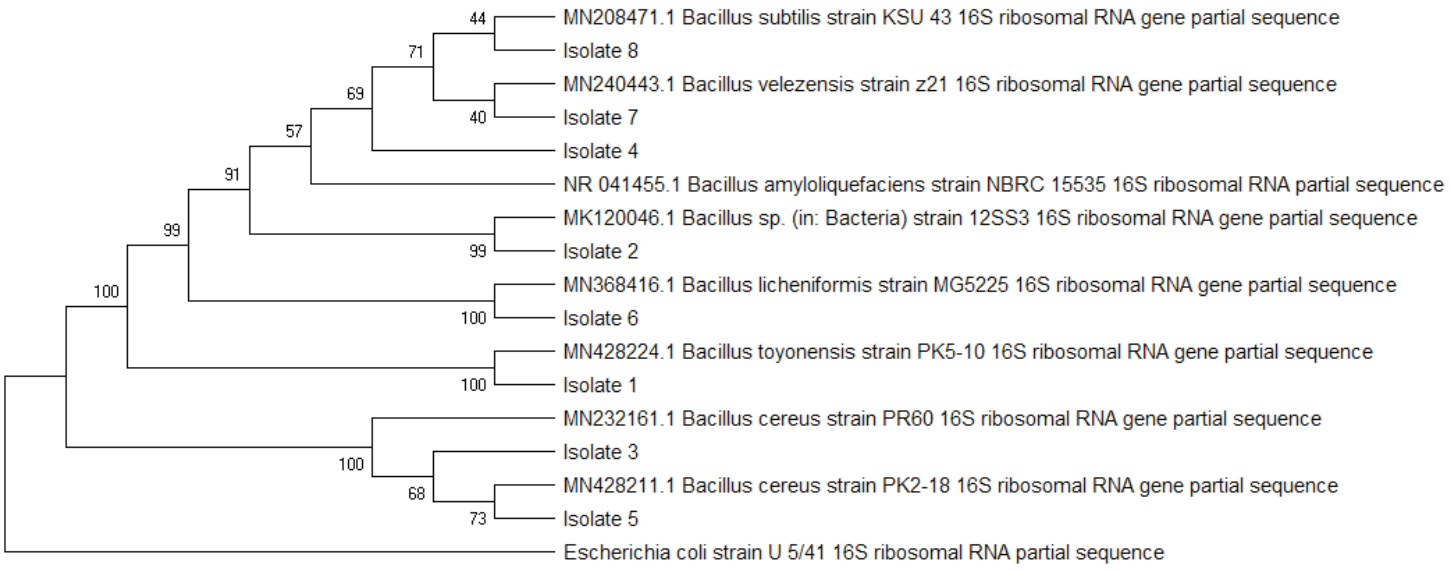


Figure 1. Construction of phylogenetic tree based on 16S rRNA gene sequencing by neighbor joining method (Mega X).
The scale bar represents 0.02 substitutions per nucleotide position.

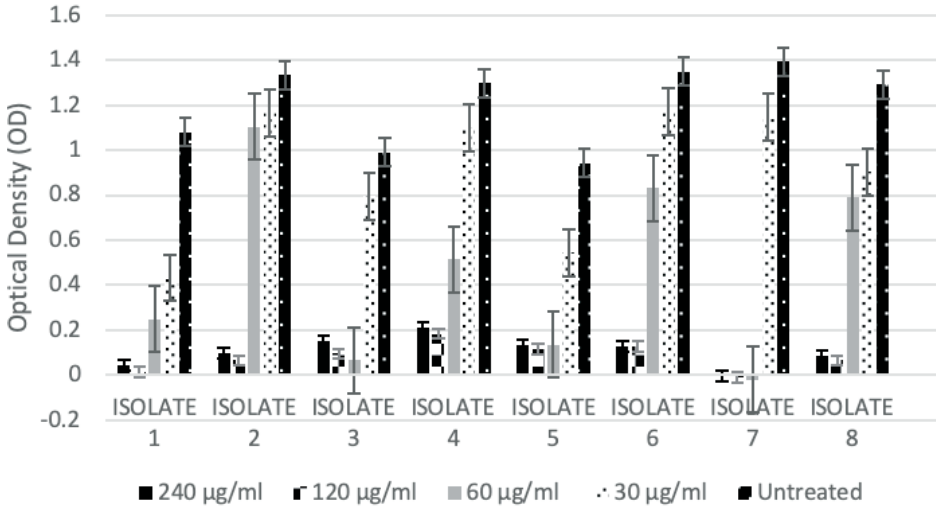


Figure 2. Dose response curves for the antimicrobial activity of thirteen isolates treated with acetone extracts of *Hypogymnia physodes* at the certain concentrations of 240, 120, 60 and 30 µg/ml at 20th hour. Data are shown as optical absorbance over OD 600 nm.

on raw skin and chrome-tanned leather samples against *Bacillus* species.[29] But, there is no study for the evaluation of lichen extracts against isolated bacteria from soak liquor samples. In this respect, the antibacterial effect of lichen species *H. physodes, E. divaricata, P. furfuracea* and *Usnea sp.* against these isolates which were assigned to several *Bacillus* species were evaluated. Acetone was preferred used as a solvent for the extraction of lichen samples in order to provide for wide-ranging extraction of the polar and semipolar constituents that may have potential bioactive properties.

According to results, *H. physodes* acetone extracts totally killed *Bacillus amyloliquefaciens* and had considerably high suppressive effect on the growth of other tested bacteria at the concentrations of 120 and 240 µg/ml. The inhibition percentage for *H. physodes* extracts against *B. toyonensis* (Isolate 1) was recorded as 96.1 and 98.6% at the concentrations of 240, and 120 µg/ml, respectively. Also, the acetone extracts of *H. physodes* had antibacterial effects on *B. mojavensis* (Isolate 2), *B. cereus* (Isolate 3 and 5), *B. velezensis* (Isolate 4), *B. licheniformis* (Isolate 6), and *B. subtilis* (Isolate 8) at the concentrations of 240 and 120 µg/ml. At 60 µg/ml of *H. physodes* extracts, there was also antibacterial effect against *B. toyonensis* (77.1%) and *B. cereus* (93.4%) when compared to the other species. 30 µg/ml of *H. physodes* extracts did not show any efficacy against all *Bacillus* species tested in this study. Data were given in Figure 2. The percentages of bacterial growth inhibition were included in Table II.

The acetone extracts of *E. divaricata* at the concentrations of 240, 120, and 60 µg/ml had significantly antibacterial effects in all tested isolates. *E. divaricata* acetone extracts totally killed *B. toyonensis*, *B. mojavensis, B. amyloliquefaciens, and Bacillus subtilis* at the concentrations of 60, 240, 60 and 240 µg/ml (100%), respectively. Furthermore, these extracts had almost significant antibacterial efficacies on *B. cereus, B. velezensis, B. licheniformis* (96.3-99.4%) at the concentrations of 240 µg/ml. At 30 µg/ml of *E.divaricata* extracts

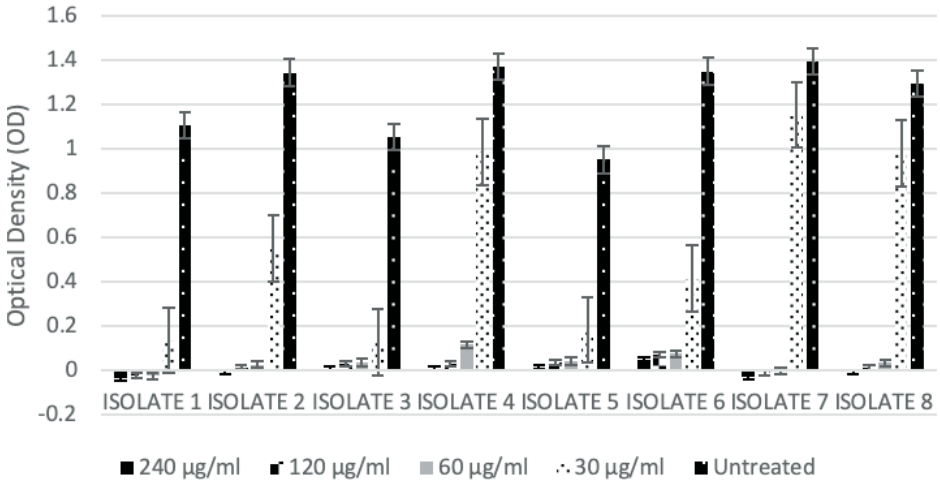


Figure 3. Dose response curves for the antimicrobial activity of thirteen isolates treated with acetone extracts of *Evernia divaricata* at the certain concentrations of 240, 120, 60 and 30 µg/ml at 20th hour. Data are shown as optical absorbance over OD 600 nm.

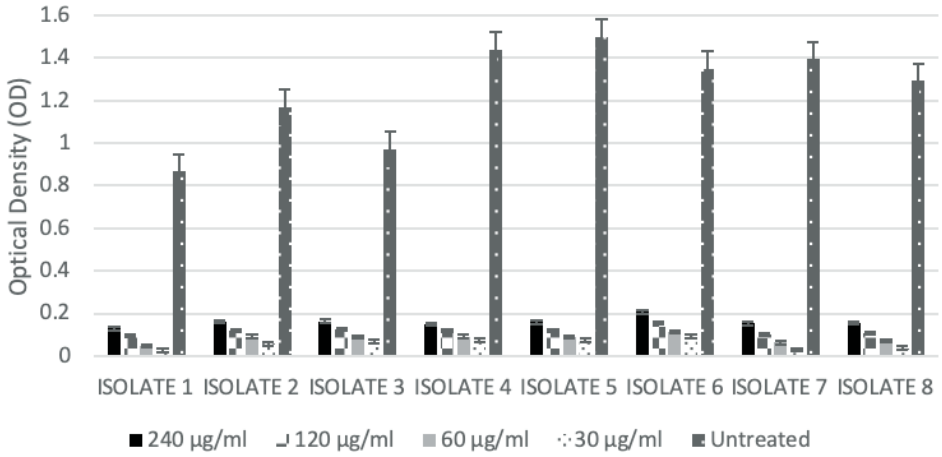


Figure 4. Dose response curves for the antimicrobial activity of thirteen isolates treated with acetone extracts of *Usnea sp.* at the certain concentrations of 240, 120, 60 and 30 µg/ml at 20th hour. Data are shown as optical absorbance over OD 600 nm.

was effective only on *B. toyonensis* (Isolate 1) and *B. cereus* (Isolate 3). The growth of *B. mojavensis* (Isolate 2), *B.licheniformis* (Isolate 6) and *B. cereus* (Isolate 5) were determined to be slightly inhibited by the extracts of *E. divaricata* extracts at 30 µg/ml. Data were given in Figure 3. The percentages of bacterial growth inhibition were included in Table II.

Similarly to the acetone extracts of *E. divaricata*, significant antibacterial effects of *Usnea sp.* in all tested isolates. However, *Usnea sp.* extracts had antibacterial activity even at 30 µg/ml in all isolates whereas *E. divaricata* had significant inhibitory effect against only two species (87.5-87.8%) in that concentration. *Usnea sp.* acetone extracts were all successful in inhibiting bacterial growth of tested bacteria. *Bacillus* species were considerably inhibited or almost killed at a percentage of 84.6-97.9 by the extracts. *Usnea sp.* acetone extracts had inhibitory effect on *Bacillus* species even at the 30 µg/ml concentration with inhibition rate over 90 % except *B.cereus* (86.6%). Data were given in Figure 4. The percentages of bacterial growth inhibition were included in Table II.

P. furfuracea extracts were also tested against isolated *Bacillus* strains. These extracts were not effective at the concentrations of 240 and 120 µg/ml to kill the bacteria but slightly inhibited the growth of them. The acetone extracts of *P. furfuracea* had a great inhibitory effect on the bacterial growth of *B. toyonensis, B. mojavensis*, *B. cereus, B. velezensis, B. licheniformis, B. amyloliquefaciens, Bacillus subtilis* only at the concentration of 240 µg/ml. In two *Bacillus* strains (*B. cereus* and *B. amyloliquefaciens*), 120 µg/ml of *P. furfuracea* extracts had similar antibacterial effect as in 240 µg/ml. Data were given in Figure 5. The percentages of bacterial growth inhibition were included in Table II.

Similarly to these data, the antibacterial effects of tested lichen species in the present study were reported in the literature against

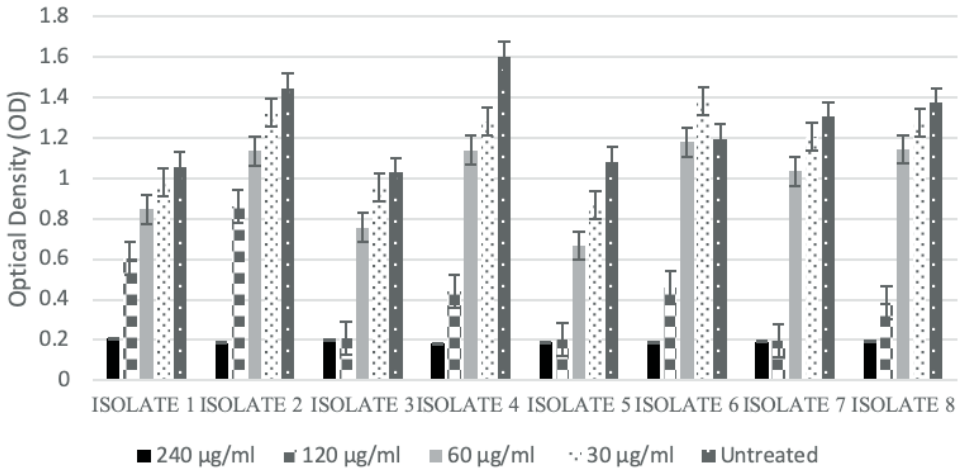


Figure 5. Dose response curves for the antimicrobial activity of thirteen isolates treated with acetone extracts of *Pseudevernia furfuracea* at the certain concentrations of 240, 120, 60 and 30 µg/ml at 20th hour. Data are shown as optical absorbance over OD 600 nm.

Table II

The percentage of bacterial growth inhibition for tested lichen extracts

	ISOLATE 1 ***Bacillus toyonensis***	ISOLATE 2 ***Bacillus mojavensis***	ISOLATE 3 ***Bacillus cereus***	ISOLATE 4 ***Bacillus velezensis***	ISOLATE 5 ***Bacillus cereus***	ISOLATE 6 ***Bacillus licheniformis***	ISOLATE 7 ***Bacillus amyloliquefaciens***	ISOLATE 8 ***Bacillus subtilis***
Pseudevernia furfuracea								
240 µg/ml	80.29	87.05	80.24	88.65	82.49	84.31	85.22	86.05
120 µg/ml	42.70	40.36	79.38	72.44	81.26	61.56	84.83	71.84
60 µg/ml	19.84	21.49	26.38	28.85	38.52	1.37	20.63	16.85
30 µg/ml	6.93	8.41	7.25	19.85	19.85	-15.53	7.60	7.31
Hypogymnia physodes								
240 µg/ml	96.1	92.7	84.5	83.8	86.1	90.3	100	93.4
120 µg/ml	98,6	95,1	90,3	85.9	87.8	90.5	100	94.8
60 µg/ml	77.1	17.2	93.4	60.2	85.7	38.2	100	39.0
30 µg/ml	59.9	12.5	19.8	15.3	42.2	13.2	17.7	30.1
Evernia divaricata								
240 µg/ml	100	100	99.2	99.4	97.8	96.3	100	100
120 µg/ml	100	98.8	97.0	97.8	93.8	94.5	100	98.8
60 µg/ml	100	98.0	96.4	91.3	93.0	94.3	100	97.3
30 µg/ml	87.5	58.9	87.8	28.0	69.6	69.2	17.4	24.2
Usnea sp.								
240 µg/ml	85.1	86.3	83.3	89.7	71.2	84.6	89.0	87.9
120 µg/ml	88.8	89.9	87.0	91.7	78.0	88.7	92.6	91.7
60 µg/ml	94.3	92.1	90.8	93.6	83.7	91.6	95.5	94.5
30 µg/ml	97.1	95.1	92.8	95.0	86.6	93.1	97.9	97.2

various *Bacillus* species. The methanol extracts of *E.divaricata* and *P. furfuracea* collected from Kastamonu province, Turkey were reported to have antibacterial efficacies against *B. megaterium* in a previous study.[47] It has been also reported that *E. divaricata* extracts were successful against *B. subtilis.*[48] *E. divaricata* extracts were also effective against *B.subtilis* in the present study. Rankovic et al. (2009) reported antimicrobial activities of *H. physodes* acetone and methanol extracts against *B. mycoides* and *B. subtilis.*[49] The extracts of several lichens were known to have more prominent effects against Gram positive bacteria in the literature. In this respect, further detailed studies have to be performed against Gram negative bacteria obtained from soak liquor samples.

Conclusion

To our knowledge, there is no study for the evaluation of *H. physodes, E. divaricata, P. furfuracea* and *Usnea sp.* against several *Bacillus* species isolated from different soak liquor samples. The antibacterial effects of *H. physodes, E. divaricata, P. furfuraceae* and *Usnea* sp. acetone extracts against *B. toyonensis, B. mojavensis, B. subtilis, B. amyloliquefaciens, B. velezensis, B. cereus,* and *B. licheniformis* was demonstrated in this study. Since antimicrobial agents may not always have the expected effect, novel agents must be investigated for the reason of bacterial resistance problems against utilized antimicrobial agents in the soaking process. Lichens may be preferred for ecological and toxicological aspects. In this respect, this study may provide an alternative approach to the leather industry to overcome bacterial resistance to the antibacterial agents. Taken together, the extracts of *H. physodes, E. divaricata, P. furfuraceae* and *Usnea* sp. must be investigated in detail for their chemicals and this/these potential chemical(s) may be utilized in the leather industry alone or in combination with antibacterial agents against antibacterial-resistant bacteria.

Acknowledgement

I express very profound gratitude to Professor Meral Birbir for her tremendous support and continuous encouragement throughout my academic career. I am thankful to Assoc. Prof. Nuzhet Cenk Sesal for his great guidance and valuable comments in every aspect of the study. The author is also grateful to Assoc. Prof. Gülsah Cobanoglu Ozyigitoglu for taxonomic identification of lichen species tested.

References

1. Polkade, A.V.; Molecular and Phylogenetic Studies on Microbial Diversity of Raw Buffalo Hide to be used in Leather Manufacturing. Doctorate Thesis, 2007.
2. Bailey, D.G., Birbir, M.; A Study of the Extremely Halophilic Microorganisms Found on Commercially Brine-cured Cattle Hides. *JALCA* **88**, 285-293, 1993.
3. Orlita, A.; Microbial Biodeterioration of Leather and Its Control: A Review. *International Biodeterioration & Biodegradation* **53,** 157-163, 2004.
4. Oppong, D., Bryant, S., Rangarajan, R., Steele, S., Radwell, D., and Hyllengren, L.; Application of Molecular Techniques to Identify Bacteria Isolated From the Leather Industry. *JALCA* **101,** 140-145, 2006.
5. Caglayan, P., Birbir, M., Sánchez-Porro, C., Ventosa, A., and Birbir, Y.; Investigation of Moderately Halophilic Bacteria Causing Deterioration of the Salted Sheep and Goat Skins and Their Extermination via Electric Current Applications. *JALCA*. **113** (2), 1, 2018.
6. Anderson, H.; The Reddening of Salted Hides and Fish. *Appl. Environ. Microbiol.* **2**, 64-69, 1954.
7. Haines, M.B.; Quality Rawstock. *JALCA* **66**, 164-173, 1984.
8. Bailey, D.G. and Birbir, M.; A Study of the Extremely Halophilic Microorganisms Found on Commercially Brine-Cured Cattle Hides. *JALCA* **88**, 285-293, 1993.
9. Birbir, M., and Ilgaz, A.; Isolation and Identification of Bacteria Adversely Affecting Hide and Leather Quality. *J. Soc. Leather Technol. Chem.* **80**, 147-153, 1996.
10. Vreeland, R.H., and Bailey, D.G.; Methods of Using Bile Salt to Inhibit Red Heat in Stored Brine Cured Hides and Skins. Patent Number 5945027, Docket number 22497, Serial Number 8906333, Agricultural Research Service, 1999.
11. Bailey, D.G, and Birbir, M.; The Impact of Halophilic Organisms on the Grain Quality of Brine Cured Hides. *JALCA* **91**, 47-51, 1996.
12. Kallenberger, W.E.; Halophilic Bacteria in Hide Curing. Ph.D. Thesis, Division of Graduate Studies and Research of the University of Cincinnati, Department of Basic Science Tanning Research of The Collage of Arts and Science, 1985.
13. Bitlisli, B.O., Karavana, H.A., Basaran, B., Sarı O., Yasa, I., Birbir, M.; The Effect of Conservation Defects on the Suede Quality of Double-face. *JALCA* **99**, 494-501, 2004.
14. Akpolat, C., Ventosa, A., Birbir, M., Sánchez-Porro, C., and Caglayan, P.; Molecular Identification of Moderately Halophilic Bacteria and Extremely Halophilic Archaea Isolated from Salted Sheep Skins Containing Red and Yellow Discoloratios. *JALCA* **110** (7), 211-220, 2015.
15. Caglayan, P., Birbir, M., Ventosa, A., and Sánchez-Porro, C.; Characterization of Moderately Halophilic Bacteria from the Salt-pack Cured Hides. *J. Soc. Leather Technol. Chem.* **99** (5), 250-254, 2015.
16. Berber, D., and Birbir, M.; Determination of Major Problems of Raw Hide and Soaking Process in Leather Industry. *International Journal of Advances in Engineering and Pure Sciences*, **31** (1), 47-52, 2019.
17. Baird, B.E., Lucia, L.M., Acuff, G.R., Haris, K.B., Sawell, J.W.; Beef Hide Antimicrobial Interventions As a Means of Reducing Bacterial Contamination. *Meat Science* **73**, 245-24, 2006.
18. Berber, D., Birbir, M., and Hacioglu, H.; Efficacy Assessment of Bactericide Containing Didecyldimethylammonium Chloride on Bacteria Found in Soak Liquor at Different Exposure Times. *JALCA* **105** (11), 354-359, 2010.
19. Birbir, Y., Uğur, G., and Birbir, M.; Inactivation of Bacterial Population in Hide-Soak Liquors via Direct Electric Current. *Journal of Electrostatics*, **66** (7-8), 355-360, 2008.
20. Russell, A.D.; Principles of Antimicrobial Activity and Resistance, in: S.S. Block (Ed.), Disinfection, Sterilization and Preservation, 5th Edition Lippincott Williams and Wilkins, Philadelphia, 31-55, 2001.
21. Madigan, M.T., Martinko, J.M., Dunlop, P.V., and Clarck, D.P. In: Brock Biology of Microorganisms, 11th Edition, Pearson Prentice Hall, Pearson Education, Inc., Upper Saddle River, NJ, 274–276, 2006.
22. Vivian, G.W.; The Preservation of Hides and Skins Against Bacterial Damage. *JALCA* **64** (10) 489-500, 1969.
23. Birbir, M., Elevi, R., Sesal, C., Vreeland, R. H., Oren, A.; Characteristics of Extremely Halophilic Bacteria Isolated from the Kaldırım and Kayacık Salterns of Sereflikoçhisar Salt Lake in Türkiye. International Conference on Halophilic Microorganisims, Sevilla, Spain, September, 23-27, 2001.
24. Weiss, E.F., Thornton, R.L.; Growth and Control of Microbiological Activity during Hide Curing Process. Buckman Laboratories Inc. 18, 1984.
25. Cremieux, A.; Freney, J.; Davin-Regli, A.; Methods of Testing Disinfectants, in: S.S. Block (Ed.), Disinfection, Sterilization and Preservation, 5th Edition Lippincott Williams and Wilkins, Philadelphia, 1305- 1327, 2001.
26. Cengiz, N.; Effects of the Solvents Used In Tanning on Environment and Workers Health. *Kocatepe Tıp Dergisi* **3**, 9-21, 2002.
27. Vijayalakshmi, K., Judith, R., and Rajakumar, S.; Novel Plant Based Formulations for Short Term Preservation of Animal Skin. *J. Sci. Ind. Res. India* **68**, 699-707, 2009.
28. Bitlisli, B.O., Yasa, I., Aslan, A. Cadirci, B. H., and Basaran, B.; Phsysical and Antibacterial Characteristics of *Aloe Vera* Treated Split Suede Leather. *JALCA* **105** (2), 34-40, 2010.
29. Türkan, M.F.; Aslan, A.; Yapıcı, A. N., Yapıcı, B.M., and Bilgi, S.T.; Assessment of Antimicrobial Activity of Natural Leathers Treated with *Pseudevernia furfuracea* (L.) Zopf extracts. *Tekstil ve Konfeksiyon* **23** (2), 176-180, 2013.
30. Singh, V.; Text Book of Botany Diversity of Microbes & Cryptogams, Rastogi Publications 373, 2014.
31. Nash III, TH.; Lichen Biology. Cambridge University Press, 303, 2008.
32. Molnár, K., Farkas, E.; Current Results on Biological Activities of Lichen Secondary Metabolites: a review. *Zeitschrift für Naturforschung C* **65** (3-4): 157-173, 2010.
33. Lobakova, E.S., Smirnov, I.A.; Advances in Applied Biotechnology: Experimental Lichenology. InTech 2012.
34. Paudel, B., Bhattarai, H. D., Lee, J. S., Hong, S. G., Shin, H. W., and Yim, J.H.; Antibacterial Potential of Antarctic Lichens against

Human Pathogenic Gram-positive Bacteria. *Phytotherapy Research* **22** (9), 1269-1271, 2008.

35. Çobanoğlu, G., Sesal, C., Gökmen, B., and Çakar, S.; Evaluation of the Antimicrobial Properties of Some Lichens. *South Western Journal of Horticulture* **1** (2), 153-158, 2010.
36. Ranković, B., Ranković, D., Kosanić, M., and Marić, D.; Antioxidant and Antimicrobial Properties of the Lichens *Anaptychya ciliaris, Nephroma parile, Ochrolechia tartarea* and *Parmelia centrifuga*. *Central European Journal of Biology*, **5** (5), 649-655, 2010.
37. Sweidan, A., Chollet-Krugler, M., Sauvager, A., Van de Weghe, P., Chokr, A., Bonnaure-Mallet, M. and Bousarghin, L.; Antibacterial Activities of Natural Lichen Compounds against *Streptococcus gordonii* and *Porphyromonas gingivalis*. *Fitoterapia* **121**, 164-169, 2017.
38. Harley, J.P. and Prescott, L.M.; Laboratory Exercises in Microbiology, 5th ed. The McGraw-Hill Companies, New York, NY, 2002.
39. Caglayan, P., Birbir, M., Sánchez-Porro, C., Ventosa, A.; Detection of Industrially Potential Enzymes of Moderately Halophilic Bacteria on Salted Goat Skins. *Turk. J. Biochem*. 43, 312-322, 2018.
40. Saitou, N., Nei, M.; The Neighbor-joining Method: A New Method for Reconstructing Phylogenetic Trees. *Mol. Biol. Evol.* **4**, 406-425, 1987.
41. Frank, J.A., Reich, C.I., Sharma, S., Weisbaum, J.S., Wilson, B.A., Olsen, G.J.; Critical Evaluation of Two Primers Commonly Used for Amplification of Bacterial 16 S rRNA genes. *Appl. Environ. Microbiol.* 74, 2461–2470, 2008.
42. Rangarajan, R., Didato, T.D. and Bryant, S.; Measurement Of Bacterial Populations In Typical Tannery Soak Solutions by Traditional and New Approaches. *JALCA* **98**, 477-485, 2003.
43. Yilmaz, E., and Birbir, M.; Characterization of Halotolerant Bacillus Species Isolated from Salt Samples Collected from Leather Factories in Turkey. *JALCA* **114** (4), 2, 2019.
44. Wood, J. T.; The Bacteriology of the Leather Industry. *Journal of the Society of Chemical Industry*, **29** (11), 666-672, 1910.
45. Venkatesan, R.A., Nandy, S.C., Sen, S.N.; Effect of Storage and Pretanning Operation on the Bacterial Flora and Its Population on Goat Skin. *Leather Science*, **17** (12), 395-404, 1970.
46. Pfleiderer, E., and Reiner, R.; Microorganisms in Processing of Leather in Biotechnology, Rehm, H.J.; Reed, G.Eds; VCH Weinheim, Germany, 66, 729-743, 1988.
47. Altuner, E. M., Ceter, T., Demirkapi, D., Ozkay, K., Hayal, U., and Eser, G.; Investigation on Antimicrobial Effects of Some Lichen Species Collected from Kastamonu region. *Communications Faculty of Sciences University of Ankara Series C: Biology* 23 (1-2), 21-31, 2011.
48. Yuan, C. H. A. O., Zhang, X. J., Du, Y. D., Guo, Y. H., Sun, L. Y., Ren, Q., and Zhao, Z. T.; Antibacterial Compounds and Other Constituents of *Evernia divaricata* (L.) Ach. *J Chem Soc Pak*, *32* (2), 189-193, 2010.
49. Ranković, B., Mišić, M., and Sukdolak, S.; The Antimicrobial Activity of Substances Derived from the Lichens *Physcia aipolia, Umbilicaria polyphylla, Parmelia caperata* and *Hypogymnia physodes*. *World Journal of Microbiology and Biotechnology* **24** (7), 1239-1242, 2008.

Comparison of the Sustainability of the Vegetable, Wet-White and Chromium Tanning Processes through the Life Cycle Analysis

by

Anna Bacardit*, Felip Combalia, Joaquim Font and Grau Baquero

A3 Leather Innovation Center. Escola Politècnica Superior (EPS). Departament d'Informàtica i Enginyeria Industrial. Universitat de LLeida (UdL). Pla de la Massa,8. 08700 Igualada, Spain.

Abstract

Public concerns about the quality of life of human beings as well as the quality of natural environments and ecosystems have led to the increasing importance of sustainability for governments and for all industries, including the leather industry. In this context, more "ecological" leather goods are being demanded. It should be noted that this concept is often used without a scientific study to support it.

This concept of sustainable or ecological product in the field of leather goods, are mainly associated with chrome-free tanneries (that is vegetable and wet-white), but this association should be backed up or dismissed by scientific evidence. A complete scientific study is required, which takes into account the different stages of leather production, including also the treatment of water and by-products, analyzing in a scientific and systematic way the environmental impact of each of these tanning processes.

This study focuses on the life cycle analysis of the three basic tanning processes: chrome, vegetable and wet-white leather production. It will focus on European manufacturing to have reliable data and reduce uncertainty.

In the tanning stage, the process with the greatest impact is the vegetable one, to highlight its high impact on global warming at 100 years (GWP100). This result is due to the energy necessary for the production of mimosa and quebracho in addition to the energy for the processing of the leather in the drums.

Wet white tanning with glutaraldehyde has lowest environmental results than vegetable tanning.

The chrome tanning process (wet blue) also stands out for its reduced environmental impact.

Subsequently, to have a global view of the entire production, the LCA of the post-tanning stages must be performed to evaluate the impact of each of the systems studied.

Introduction

The life cycle analysis (LCA) is a methodological tool that allows us to evaluate the environmental loads associated with a product, process or activity. To do this, the raw materials, chemicals and energy used as well as the emissions to the environment are identified and quantified in order to quantify the impact of the use of these resources and those emissions to subsequently determine environmental improvement strategies focused on minimization.

This tool takes into account stages of extraction and processing of materials, production, transport and distribution, use, reuse and maintenance, recycling and final disposal.

Its application is standardized by the ISO, International Organization for Standardization, through the following standards:

- UNE EN ISO 14040: 2006. Environmental management. Life cycle analysis. Principles and frame of reference.
- UNE EN ISO 14044: 2006. Environmental management. Life cycle analysis. Requirements and guidelines.

LCA is used in the application of eco-design in products and services, in the elaboration of the criteria for compliance with ecological labels, as a source of information for the implementation of environmental management systems, in comparative studies between similar products (different origin, composition but same function) and any other case related to obtaining information about the environmental behavior of a product or service.

In this work, this tool is used to compare the environmental impact of different tanning processes: vegetable, wet-white (WW) and chrome (wet-blue, WB).

Public concerns about the quality of life of human beings as well as the quality of natural environments and ecosystems have led to the increasing importance of sustainability for governments and for all industries, including the leather industry. In this context, more

*Corresponding author email: anna.bacardit@udl.cat

Manuscript received November 4, 2019, accepted for publication December 19, 2019.

"ecological" leather goods are being demanded. It should be noted that this concept is often used without a scientific study to support them.

This concept of sustainable or ecological product in the field of leather goods, are mainly associated with chrome-free tanneries (that is vegetable and wet-white), but this association should be backed up or dismissed by scientific evidence. A complete scientific study is required, which takes into account the different stages of hide production, including also the treatment of water and by-products, analyzing in a scientific and systematic way the environmental impact of each of these tanneries.

The final objective of this work will be the dissemination of the results of the study in a scientific and pedagogical way.

In the literature comparison works between WB-WW tanning technologies can be found, with results that indicate trends, but always with nuances and uncertainties.

According to Xu et al.,[1] wet-white tanning compared to chrome tanning, has a lower energy consumption so the impact on global warming potential (GWP) is lower. Even so, it should be objected that wastewater treatment should be taken into account.

In a 2016 study[2] it is identified that chromium derivatives have a problem of depletion of abiotic resources and - at the same time - that the global warming potential (GWP) in the case of wet-white tanning may be higher if some factors are excluded.

As indicated by Laurenti et al.,[3] the variability in the results obtained through a study-survey of several tanneries regarding the consumption of water and energy (electrical and thermal) demonstrates that the data for the tanning process should be used with caution in a context of decision making. The need for a more specific study, focusing on comparable products and specific productions, is detected since, according to the same author, the location and production methods greatly influence the use of water and energy, highlighting the importance of wastewater treatment in the own tannery, if it exists.

It should be noted that in the studies found in the literature, it has not been considered that part of the chromium used in the stores would be a byproduct of the pharmaceutical industry. For this reason, in an environmental study the use of a byproduct can greatly affect the comparative result.

A comparative study has not been found a priori that includes WW, WB and vegetable tanning but due to the own experience in different studies,[4-6] it can be affirmed that vegetable tanning has an energy consumption and a GWP greater than the other types of process. They should be studied in other environmental impacts and take into account the stages to be compared for a complete comparative study.

According to the compilation of Dixit et al.,[7] the negative impact associated with the leather industry requires the so-called Green Technology, which can be divided into two methods: waste reduction or improvement in technology that reduces the toxicity of waste and wastewater treatment and solid waste processing. These concerns are not new, for example, there are studies of recovery of chromium salts from wastewater 20 years ago.[8]

Unlike the basic material and energy balances, the LCA approach takes into account the production of the chemicals that are used, as well as the emissions that are generated. This broader vision of the process leads to a more detailed environmental assessment, quantitatively measuring the effect of chemicals and energy involved.[5]

It should be noted that the use of incomplete data, data from technologies experienced only in pilot tests but not validated in the industry, or the omission of any part of the process (or subprocess) can lead to biased comparative results. For this reason, it is preferable to specify in product, type of tanning and stages of the process to be compared in order to obtain specific and unquestionable results.

Experimental – Scope and limits of the system

The study focuses on the production of leather for bovine leather items for footwear and leather goods. It will focus on European manufacturing to have reliable data and reduce uncertainty.

It should be considered that a comparative generalist study of tanning processes covers a huge number of variables and it is necessary to begin by limiting its scope.

The comparative result will show the impact of producing leather considering the products used and their origin and production, as well as emissions to the environment through standardized impact indices.

Production depends on the type of factory and its location, but above all it is important to define the product that is produced and for this, this comparative study will focus specifically on the processes of:

- WB: Chrome tanning process (wet-blue)
- WW: Wet-white tanning process (by glutaraldehyde and by tara)
- Vegetable: Vegetable tanning process

Chrome tanning is the most widely used globally, due to its speed and savings in water and products, which make it very economically competitive. According to the latest report of best available techniques (BAT, Best Available Techniques), 80-90% of the world would use chromium III salts in their tanning processes.[9]

It should be taken into account that the first stages of the tanning process (previous operations and beamhouse process) are common in the different processes considered in this study. In the stages of tanning and post-tanning it is where the main differences between the different tanning processes are found. However, in this study

the post-tanning process and the finishing stage have not been considered in this study.

Each product used has a different environmental impact associated due to its own production and the treatment required by the waste generated by its use (in this particular case mainly wastewater), an effect that will be taken into account in the study of life cycle analysis.

Wastewater treatment is considered a necessity in a study of this nature. It is possible to consider the process for each type of wastewater, considering the energy, and products necessary to obtain water with the necessary characteristics for its return to the environment without negatively affecting it.

In addition to the treatment of wastewater baths, the specific risks of each process must be taken into account in accordance with the corresponding wastewater treatment.

This study includes this treatment, however, the relative low impact in the results shows no influence from the wastewater treatment on the conclusions.

Experimental – Inventory

The inventory includes the inputs and outputs in each process of energy and matter, which will be considered generation, production, transport and management of waste generated. Tanning formulas considered conventional for each process are reflected in this study.

The specific data considered in the reference formulation of each of the processes considered are those shown in the tables:

Table I: Chrome tanning process (wet-blue)

Table II: Wet-white tanning process using glutaraldehyde

Table III: Wet-white tanning process incorporating tara

Table IV: Vegetable tanning process using mimosa and quebracho

Considerations for all processes in this initial study:

- It is considered a maximum use of tanning products, assuming optimal process conditions in terms of time, temperature and application of the products.
- A medium thickness (3.5mm) for split hides is selected for the initial comparison between processes, which involves similar penetration times and fixation of the tanning products. In the case of tanning with vegetable extracts, for articles of greater thickness (eg, sole, belt) it will be necessary to increase the amount of extracts and consequently the processing time for complete penetration and fixation.
- Water heating for the processes was obtained by means of a gas boiler. The thermal energy input into the system has not been considered, but it is considered that the temperature is maintained within the drum due to the mechanical effect itself.
- In the case of the wet white process, the tanning stage will be referred to as pre-tanning since it is essential to retan the wet white hide obtained for its complete stabilization. In the other tanneries, post-tanning confers certain characteristics, but the hide is already stabilized with the tanning stage.
- If we do not have in the database to calculate a specific product, a product with similar characteristics (called proxies) has been selected.

Table I

Inputs and outputs for the chrome tanning process (wet blue)

TANNING			
Inputs			
Pickled hide	Mass	1000	kg
Chromium salt 33°Sch	Mass	65	kg
Water 20°C	Mass	500	kg
Energy for drum (1565 kg, 120 min)	Electric energy	169	MJ
Thermal energy (agua 20 → 45°C)	Thermal energy[a]	96.86	MJ
Magnesium Oxide	Mass	3.5	kg
Fungicide	Mass	1	kg
Energy for drum (1569.5 kg, 480 min)	Electric energy	678.02	MJ
Outputs			
Wet blue hides	Mass	1069.5	kg
Chromium tanning process wastewater	Mass	500	kg

[a]Net calorific value.
Remark: kg = kilograms; min = minutes; MJ = megajoules.

Table II

Inputs and outputs for the wet-white tanning process using glutaraldehyde

PRETANNING			
Inputs			
Pickled hide	Mass	1000	kg
Water 20°C	Mass	50	kg
NaCl	Mass	3	kg
Energy for drum (1053 kg, 15 min)	Electric energy	14.22	MJ
Glutaraldehyde	Mass	25	kg
Energy for drum (1078 kg, 210 min)	Electric energy	203.74	MJ
Phenolic syntan	Mass	50	kg
Energy for drum (1128 kg, 300 min)	Electric energy	304.56	MJ
Water 20°C	Mass	300	kg
Fungicide	Mass	1	kg
Energy for drum (1428 kg, 20 min)	Electric energy	25.70	MJ
Outputs			
Wet white hides	Mass	1078	kg
Wet-white tanning process wastewater	Mass	350	kg

Remark: kg = kilograms; min = minutes; MJ = megajoules.

Table III

Inputs and outputs for the wet-white tanning process using glutaraldehyde and tara

PRETANNING			
Inputs			
Pickled hide	Mass	1000	kg
Water 20°C	Mass	50	kg
NaCl	Mass	3	kg
Energy for drum (1053 kg, 15 min)	Electric energy	14.22	MJ
Tara	Mass	90	kg
Phenolic syntan	Mass	20	kg
Ferric pyrophosphate	Mass	40	kg
Sulfited oil	Mass	20	kg
Energy for drum (1223 kg, 480 min)	Electric energy	528.34	MJ
Formic acid	Mass	40	kg
Energy for drum (1563 kg, 240 min)	Electric energy	337.61	MJ
Drain			
Water 20°C	Mass	300	kg
Fungicide	Mass	1	kg
Energy for drum (1447 kg, 20 min)	Electric energy	26.05	MJ
Outputs			
Wet white with tara hides	Mass	1147	kg
Wet white with tara process wastewater	Mass	960	kg

Remark: kg = kilograms; min = minutes; MJ = megajoules.

Table IV

Inputs and outputs for the vegetable tanning process

TANNING			
Inputs			
Pickled hide	Mass	1000	kg
Water 20°C	Mass	600	kg
Phenolic syntan	Mass	50	kg
Energy for drum (1650 kg, 60 min)	Electric energy	89.10	MJ
Mimosa extract	Mass	160	kg
Energy for drum (1810 kg, 180 min)	Electric energy	293.22	MJ
Water 25°C	Mass	50	kg
Thermal energy (water 20→25°C)	Thermal energy[a]	15.94	MJ
Quebracho extract	Mass	120	kg
Sulfited oil	Mass	7,5	kg
Energy for drum (1987.5 kg, 560 min)	Electric energy	1001.70	MJ
Drain			
Water 20°C	Mass	1000	kg
Energy for drum (2226.5 kg, 25 min)	Electric energy	50.10	MJ
Fungicide	Mass	1	kg
Drain			
Outputs			
Vegetable hide	Mass	1226.5	kg
Vegetable process 1 wastewater	Mass	762	kg
Vegetable process 2 wastewater	Mass	1000	kg

[a]Net calorific value.
Remark: kg = kilograms; min = minutes; MJ = megajoules.

As can be seen, the amount of water used is much higher when using vegetable tanning products. Likewise, it should be noted that the fixation of the tanning principle in vegetable products is less than in the case of chromium salts or glutaraldehyde.

It can be also observed the consumption of electrical energy considered in terms of the load and process time, a prominent contributor of electrical consumption in tanning processes. Also highlight the need for thermal energy for the wet blue process and to a lesser extent in vegetable tanning.

Wastewater treatment energy is considered 8.41MJ/m^3 according to data from the reference Wastewater treatment plant of IDR in Igualada, which treats the effluents from the tanneries grouped in the Leather Cluster Barcelona from Igualada.

Experimental – Characteristics of the wastewater of the different processes

The characterization of the wastewater for each process is shown in Table V. The data shown are analysis of floats obtained in tests performed at A3 leather innovation center.

As it is observed, there are parameters that only affect some of the processes (Chromium and glutaraldehyde). The chromium oxide value

Table V

Comparison of the pollutant load in the tanning stage in the processes considered

Parameter	*WB Cr*	*WW Glut.*	*WW Tara*	*Vegetable*	*Units*
pH	3.8	4.3	3.8	4.5	-
Suspended matter	793	9097	7976	9105	mg/L
COD	11320	32550	25560	153600	mg O_2/L
Total Nitrogen[a]	1380	362	390	3280	mg N/L
Conductivity	11255	78315	69855	26522	µS/cm
Chromium Oxide	111	0	0	0	mg/L Cr_2O_3
Glutaraldehyde[b]	0	300	0	0	mg/L glutaraldehyde

[a]Organic and Ammoniacal Nitrogen.
[b]Estimation from experimental data.
Remark: DQO = Chemical oxygen demand; mg/L = miligrams/liter; mg O2/L = oxygen miligrams/liter; mg N/L = Nitrogen miligrams/liter; µS/cm = microsiemens/centimeter.

shown is a value corresponding to a residual bath in a process with moderate exhaustion. High float exhaustion (high use of chromium salts) can be obtained by optimizing the tanning process and consequently lower values of this parameter can be obtained. In the case of glutaraldehyde, it is an estimate based on experimental data.

In the suspended matter parameter, for the analysis shown high values are detected in the tanning using glutaraldehyde due to the phenolic syntan use. In the process of tanning using tara, it is also a high parameter due to its high content of non-soluble substances. In the case of mimosa and quebracho (considered vegetable tanning) it is also high.

Also comment on the high COD and total nitrogen values in vegetable tanning, since most of the suspended matter in this bath is organic matter.

Results

The results of the life cycle analysis are shown based on the impacts considered (impact categories). Each impact takes into account different consumptions and emissions with respect to the environment to a different extent according to pre-established scales by recognized research institutions or centers. They are collected as analysis of life cycle impacts (from LCIA, Life Cycle Impact Assessment).

In this study, the group of impact categories according to CML (baseline), version 4.4 (January 2015), developed by the Institute of Environmental Sciences of the University of Leiden, has been used. It is considered a classic method of impact evaluation, which quantifies the stages of the cause-effect chain of the process considered. The results are grouped into the so-called mid-point impact categories. Themes are common mechanisms for the impact considered. For example, the one with the most consensus among different calculation methods is the impact of global warming (GWP) associated with the topic "Climate change", expressed in kg of CO_2.

Table VI

Impact categories considered (CML baseline, v4.4, January 2015)

Impact categories	*Units*
Acidification potential – average Europe	kg SO_2 eq.
Climate change – GWP100	kg CO_2 eq.
Depletion of abiotic resources – elements, ultimate reserves	kg antimony eq.
Depletion of abiotic resources – fossil fuels	MJ
Eutrophication – generic	kg PO_4^{---} eq.
Freshwater aquatic ecotoxicity – FAETP inf	kg 1,4-dichlorobenzene eq.
Human toxicity – HTP inf	kg 1,4-dichlorobenzene eq.
Marine aquatic ecotoxicity – MAETP inf	kg 1,4-dichlorobenzene eq.
Ozone layer depletion – ODP steady state	kg CFC-11 eq.
Photochemical oxidation – high Nox	kg ethylene eq.
Terrestrial ecotoxicity – TETP inf	kg 1,4-dichlorobenzene eq.

The study also includes the impacts from the Cumulative Energy Demand (CED) impact categories, Version 2 (based on the method published by Ecoinvent (Swiss Centre for LC, Duebendorf, CH).

Table VI shows the environmental impact categories used in this preliminary study as well as the reference units with which their impact is measured.

The results corresponding to these impacts can be represented in a grouped way in a comparative radar diagram, referenced for each category of impact to the process in which there is a greater impact (100%) and graphically showing the relative impact of the other processes. The environmental results of the tanning processes considered are shown in Figure 1. The results in terms of energy

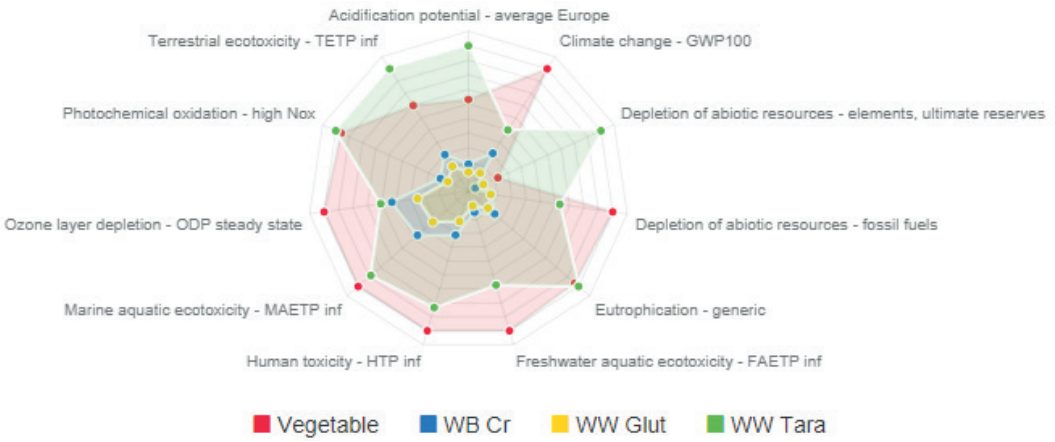


Figure 1. Comparative environmental results of the life cycle analysis of the wet blue, wet white and vegetable tanning processes

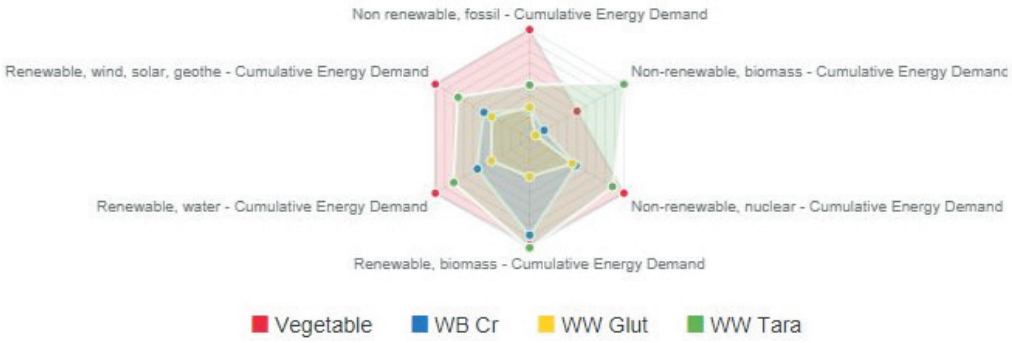


Figure 2. Comparative energy results of the life cycle analysis of the wet blue, wet white and vegetable tanning processes

demand are presented in Figure 2 in order to show basically the energy consumption according to the different origin of the products.

As can be seen in the results obtained, the vegetable process is the one with the greatest environmental impact, clearly noticeable in the high impact on global warming at 100 years (GWP100), stated as climate change in Figure 1. This result is due to the energy necessary for the production of mimosa and quebracho in addition to the energy used in drums. Wastewater treatment has slight significance as its impact is proportionally very low when comparing to other contributors. Namely, the energy contribution of wastewater treatment varies from 3 to 15 MJ depending on the considered process, representing a relative contribution of 0.5% to 1.25% in the energy consumption.

Cumulative energy demand indicators from Figure 2 show, as expected from the formulations a higher energy demand for the vegetable process and the lowest for the WW with glutaraldehyde. However, the production energy from specific products can affect this general layout. In this case, it must be highlighted that the use of tara is revealed as the cause of the comparative increase in non-renewable biomass energy demand for WW using tara process due to the use of pyrophosphate in the formulation, which uses biomass from primary forest.

The wet white tanning process using tara also has high environmental results, greater than vegetable tanning in several impacts. The main contributors are the proxies of ferric pyrophosphate and formic acid, and in some category the production of tara.

Wet white with glutaraldehyde process has the lowest environmental results according to these results.

The chrome tanning process (wet blue) also stands out for its reduced environmental impact, although it should be taken into account that the review of the processes and the validation of the selected proxies or their replacement by specific processes in the final review is pending.

Finally, bear in mind that the extension - already planned for further studies - of the system limits considering post-tanning as well as the treatment of wastewater may affect the result of the analysis.

On the other hand, highlight that it is outside the scope of this work, the life of the tanned product and its end (end-of-life).

For example, the wet white process with glutaraldehyde should also be taken into consideration, since even though it is better at the level of environmental impact in the tanning stage, the amount of products used in the post-tanning process must be increased to obtain a leather with good characteristics. In addition, it should be considered that glutaraldehyde can lead to problems in the biological scrubbers of the water treatment stages.

Conclusions

The study has been focused on the life cycle analysis of the three basic tanning processes: chrome, vegetable and wet-white leather production. Specificaly, it has been focused on European manufacturing to have reliable data and reduce uncertainty.

The comparative results have shown the environmental impacts of leather tanning taking into account the products used and their origin and production, including emissions to the environment through standardized impact indices (CML and CED).

From the results obtained, it can be stated that in the tanning stage, the process with the greatest impact is the vegetable one, to highlight its high impact on global warming at 100 years (GWP100). This result is due to the energy necessary for the production of mimosa and quebracho in addition to the energy for the processing of the leather in the drums. Wet white tanning with glutaraldehyde has lowest environmental results than vegetable tanning. The chrome tanning process (wet blue) also stands out for its reduced environmental impact.

Subsequently, to have a global view of the entire production, the LCA of the post-tanning stages must be performed to evaluate the impact of each of the systems studied.

References

1. Xu, X., Baquero, G., Puig, R., Shi, J., Sang, J., Lin, W.; Carbon footprint and toxicity indicators of alternative chromium-free tanning in China. *JALCA*, **110**(5), 130-137, 2015.
2. Shi, J., Puig, R., Sang, J., Lin, W.; A comprehensive evaluation of physical and environmental performances for wet-white leather manufacture. *Journal of Cleaner Production*, **139**, 1512-1519, 2016. https://doi.org/10.1016/j.jclepro.2016.08.120.
3. Laurenti, R., Redwood, M., Puig, R., Frostell, B.; Measuring the Environmental Footprint of Leather Processing Technologies. *Journal of Industrial Ecology*, **21**, 1180–1187, 2017. https://doi.org/10.1111/jiec.12504.
4. Bacardit, A., Baquero, G., Sorolla, S., Ollé, L.; Evaluation of a new sustainable continuous system for processing bovine leather. *Journal of Cleaner Production*, **101**, 197-204, 2015. https://doi.org/10.1016/j.jclepro.2015.04.012.
5. Baquero, G., Sorolla, S., Ollé, L., Bacardit, A.; Environmental assessment of dehydration processes for bovine leather. *JALCA*, **112** (3), 102-108, 2017.
6. Kiliç, E., Puig, R., Baquero, G., Font, J., Çolak, S., Gürler, D.; Environmental optimization of chromium recovery from tannery sludge using a life cycle assessment approach. *Journal of Hazardous Materials*, **192**(1), 393-401, 2011.
7. Dixit, S., Yadav, A., Dwivedi, P.D., Das, M.; Toxic hazards of leather industry and technologies to combat threat: a review. *Journal of Cleaner Production*, **87**, 39–49, 2015. https://doi.org/10.1016/j.jclepro.2014.10.017
8. Fabiani, C., Ruscio, F., Spadoni, M., Pizzichini, M.; Chromium(III) salts recovery process from tannery wastewaters. *Desalination* **108**, 183–191, 1997. https://doi.org/10.1016/S0011-9164(97)00026-X
9. Black, M., Canova, M., Rydin, S., Scalet, B.M., Roudier, S., Sancho, L.D.; Best available techniques (BAT) reference document for the tanning of hides and skins. Seville, Spain. 2013. https://doi.org/JRC 83005. EUR 26130 EN. ISBN 978-92-79-32947-0

Lifelines

Fatih Yalçin graduated Bachelor and Master degree of Leather Engineering Department, Ege University. He is still at same department with doctorate thesis stage. His master thesis is about diabetic footwear that produced with drug released leather. During his master stage worked in Romania. One is about patrimonial leather objects conservation and their non-destructive test methods, in Bucharest about 4 months. Other is approach of footwear engineering, expectations and requirements, in Iasi for 5 months. He worked for 4 years as R&D engineer of technical footwear factory. He dealt with safety and military shoes and Gore-Tex goods production expectation, test requirements and its qualification and its specification compliance. During that time, he was the factory responsible person at "Mine Boot" project, between Turkey Defense Industry Agency and YDS Boots. Now, he is working at Uşak University Technology Transfer Office to be bridge between leather and textile companies and University to gather scientific projects and factory expectations. He is still dealing with the leather and footwear expectations, test requirements and specification of this kind of goods and their scientific projects.

Hüseyin Ata Karavana graduated from the Leather Technology Department of Agriculture Faculty at Ege University, Turkey in 1996. He received his M.Sc. degree in Leather Technology in 2001 from Graduate School of Natural and Applied Science, Ege University. He was an Erasmus student in Department of Footwear Engineering and Hygine, Faculty of Technology, Tomas Bata University in Zlin, Czech Republic from 2006 to 2007. Hüseyin Ata Karavana received his Ph.D. degree in Leather Engineering in 2008 at Ege University, Department of Leather Engineering. His research interests are leather quality and control, footwear quality and control, leather engineering topics, footwear engineering topics, plastic composites, microencapsulation. He has published 25 research articles. He presented 65 oral and poster presentations in international and national congresses. He completed 29 international and national research projects. He has been working as an Associate Professor in Department of Leather Engineering, Engineering Faculty, Ege University in Turkey.

Seda Rençber graduated from the Ege University Faculty of Pharmacy, Turkey in 2006. She has completed her MSc thesis on the issue of "Preparation and Evaluation of Non-Steroidal Antiinflammatory Drugs Solid Dispersion" from Deparment of Pharmaceutical Technology Faculty of Pharmacy Ege University in 2009. She has completed her Ph.D. thesis on the issue of "Development and *In vitro*/*In vivo* Evaluation of New Bioadhesive Dosage Forms for Local Treatment of Fungal Infections of Oral Mucosa" from Deparment of Pharmaceutical Technology Faculty of Pharmacy Ege University in 2014. Her research interests are semi-solid drug formulations, mucoadhesive dosage formulations, nanoparticle drug delivery systems, hot-melt extruder, solid dispersions, microencapsulation, buccal and vaginal formulations and rheology. She has published 18 research articles. She presented 53 oral and poster presentations in international and national congresses. She completed 14 national research projects. She has been working as an Research Assistant in Department of Pharmaceutical Technology, Faculty of Pharmacy, Ege University in Turkey.

Sinem Yaprak Karavana received all of her degrees from Ege University, Faculty of Pharmacy, Department of Pharmaceutical Technology. She received Bachelor of Science in 1997, MSc in 2000. She has completed her Ph.D. thesis on the issue of "Preparatıon of Bıoadhesıve Benzıdamıne Hydrochlorıde Formulatıons and *In Vıtro-In Vıvo* Evaluatıon" in 2007. She has published 33 articles on topics such as drug development, bioadhesive systems, buccal drug delivery and topical delivery. She has been working as an Associate Professor in the Faculty of Pharmacy, Department of Pharmaceutical Technology at Ege University in Turkey.

Peng Liang-qiong received her Bachelor's Degree in Environmental Engineering at Sichuan Normal University in 2016. Then she received her degree of Master of Engineering in Leather Chemistry and Engineering at Sichuan University in 2019. Her research focuses on leaching of harmful substances from leather and its ecological evaluation.

Long Wen-jun is majoring in Light Chemistry Engineering at Sichuan University.

Li Yue received her Bachelor's Degree in Light Industry Biotechnology at Sichuan University in 2019.

Zhang Wen-hua, see *JALCA* 100, 432, 2005.

Shi Bi, see *JALCA* 99, 220, 2004.

Didem Berber graduated from Marmara University Atatürk Faculty of Education, Biology Department in 2001. She received MSc. degree from Pediatric Allergy-Immunology Department of Marmara University Medical Faculty in 2003 and Ph.D. degree from Department of Biology, Faculty of Arts and Sciences of Marmara University in 2010. She has been studying as post doctorate researcher in Marmara University, Department of Biology, Faculty of Arts and Sciences from 2016 up to date. She contributed to international projects (COST and other bilateral collaboration projects) on bacterial quorum sensing and biofilm inhibition. Her research topics are hide microbiology, environmental microbiology, antimicrobial agents, fungi, quorum sensing, and biofilm formation.

Anna Bacardit graduated in Chemistry Technical Engineering at the Universitat Politècnica de Catalunya (UPC) in Spain in 1994. Graduated in Management Engineering at the Universitat Politècnica de Catalunya (UPC) in Spain in 2003. She got her Ph.D. degree in Chemistry at the University of Barcelona in 2005. She received the Master in Tanning Technical Management from the Universitat Politècnica de Catalunya (Spain) in 2001. From 1995 to 1999 she worked in the Technical Laboratory at BASF-Curtex in L'Hospitalet (Spain). Since 1999 until 2018 she worked as a lecturer at the Universitat Politècnica de Catalunya (UPC) in Spain. Since 2018 she is professor at Universitat of Lleida. She is mainly involved in the development of cleaner and innovative leather processing methods.

Felip Combalia graduated in Chemistry Technical Engineering at the Universitat Politècnica de Catalunya (UPC) in Spain in 1999. Graduated in Management Engineering at the Universitat Politècnica de Catalunya (UPC) in Spain in 2006. He got his Ph.D. degree at the Universitat Politècnica de Catalunya in 2013. He received the Master in Tanning Technical Management from the Universitat Politècnica de Catalunya (Spain) in 2006. From 2000 until 2018 he worked as a lecturer at the Universitat Politècnica de Catalunya (UPC) in Spain. Since 2018 he is professor at Universitat of Lleida. He is mainly involved in the development of cleaner and innovative leather processing methods.

Joaquim Font, Ph.D. in Chemistry from the University of Barcelona, he has been professor at the Universitat Politècnica de Catalunya (UPC) in Spain from 1984 until 2018. Since 2018 he is professor at Universitat of Lleida. He has participated in various national and international research projects and has published more than 25 research papers and a book in Spanish about Testing in the Leather Industry.

Grau Baquero, Industrial Engineer from Universitat de Girona (2005). Ph.D. at Universitat Politècnica de Catalunya (2011). He has worked in the industrial food sector until 2005. From 2005 until 2018 he worked as a lecturer at Universitat Politècnica de Catalunya. Since 2019 he is professor at the Universitat de Lleida. He is mainly involved in environment and life cycle assessment research projects.

CALL FOR PAPERS

FOR THE 116th ANNUAL CONVENTION OF THE AMERICAN LEATHER CHEMISTS ASSOCIATION

Eaglewood Resort & Spa, Itasca, Illinois
June 16-19, 2020

If you have recently completed or will shortly be completing research studies relevant to hide preservation, hide and leather defects, leather manufacturing technology, new product development, tannery equipment development, leather properties and specifications, tannery environmental management, or other related subjects, you are encouraged to present the results of this research at the next annual convention of the Association to be held at the Eaglewood Resort & Spa, Itasca, Illinois, June 16-19, 2020.

Abstracts are due by April 1, 2020
Full Presentations are due by June 1, 2020

They are to be submitted by e-mail to the
ALCA Vice-President and Chair of the Technical Program:

Joseph Hoefler
The Dow Chemical Company
400 Arcola Rd.
Collegeville, PA 19426
E-mail: jhoefler@dow.com

The **Abstract** should begin with the title in capital letters, followed by the authors' names. An asterisk should denote the name of the speaker, and contact information should be provided that includes an e-mail address. The abstract should be no longer than 300 English words, and in the Microsoft Word format.

Full Presentations at the convention will be limited to 25 minutes. In accordance with the Association Bylaws, all presentations are considered for publication by *The Journal of the American Leather Chemists Association*. They are not to be published elsewhere, other than in abstract form, without permission of the *Journal* Editor. For further paper preparation guidelines please refer to the *JALCA* Publication Policy on our website: leatherchemists.org

Full Presentations are to be submitted by e-mail to the *JALCA* editor:

Steven D. Lange, *Journal* Editor
The American Leather Chemists Association
E-mail: jalcaeditor@gmail.com
Mobile Phone (814) 414-5689

Scholarship Application Available

Randall L. Rowles

Applications for the Randall Rowles Memorial Scholarship are now available through the ALCA office by either writing to ALCA, 1314 50th Street, Suite 103, Lubbock, TX 79412; or by emailing to carol.adcock@ttu.edu; or by calling (806) 744-1798.

Guidelines for the scholarship have been revised to broaden the requirements for the recipient. The recipient of the scholarship shall be a relative of an ALCA member in good standing or someone proposed by an ALCA member and pursuing a post-secondary education in the sciences. A current high school or college transcript must be submitted to the ALCA office along with an application.

The scholarship shall be based on merit. The GPA of the applicant will be a significant criterion for the selection committee.

Completed applications must be received at the ALCA office no later than May 1, 2020. The selection of the recipient will be made no later than June 1, 2020. Members of the Selection Committee will be Lori Hyllengren and Nick Latona, Co-Chairs, Mike Bley, Okey Abara and Cheng-Kung Liu.

The amount of the scholarship is $500, and the recipient will be announced at the Annual Business Meeting of the Association on Thursday, June 18, 2020, at Eaglewood Resort & Spa, Itasca IL.

THE AMERICAN LEATHER CHEMISTS ASSOCIATION
1314 50th Street, Suite 103, Lubbock, Texas 79412-2940
Ph: 806-744-1798 **Fax**: 806-744-1785 **Web**: www.leatherchemists.org
Email: carol.adcock@ttu.edu

QUALITY LASTS.

Enduring results are created stitch by stitch. To achieve them, we provide leather manufacturers worldwide with products and know-how on future fashion trends. Furthermore, we pay particular attention to the best options available in terms of sustainability. Our products provide better results through superior durability. They also minimize the use of critical resources and help meet regulatory objectives. Our “Sustainable Leather Management” initiative enables us to meet responsibilities that go far beyond the supply of quality products. LANXESS quality meets the trends of tomorrow, sustainability. **leather.lanxess.com**

SUSTAINABLE
LEATHER MANAGEMENT®
BY LANXESS

QUALITY WORKS.

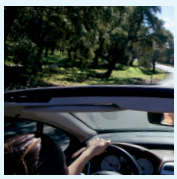
LANXESS
Energizing Chemistry

Stahl's innovations driven by sustainability

With the rise of both electric and self-driving, cars are becoming quieter and anti-squeak and rattle materials are becoming increasingly important. At the same time, improved anti-stain performance is required, because of the current trend for pale-colored car seats. Therefore, we have developed Stay Clean. This low-VOC coating technology protects pale-colored leather and vinyl surfaces against common stains, such as dye from jeans, spilled coffee and dirt. Our solution also makes surfaces low-squeak, which is a great asset as global research has shown that a squeaking car interior is one of the biggest annoyances among car owners. Another trend in car interior is the popularity of matt surfaces. Therefore, we have developed PolyMatte®. This non-squeaking solution provides a luxurious feel to the finished article in combination with flexibility and scratch and abrasion resistance. Our portfolio contains many products, varying from beamhouse products, tanning systems to finishes, duller concentrates, crosslinkers and thickeners to leveling agents, defoamers, colorants and hand modifiers. Our most sustainable option is Green PolyMatte®, which is based on rapeseed oil (20%) instead of crude oil-derived intermediates. If you would like to know what our Stahl solutions for automotive can do for your business, please visit **www.stahl.com** or contact us at: **alexander.campbell@us.stahl.com**.

If it can be imagined, it can be created

Stay Clean



Low-VOC

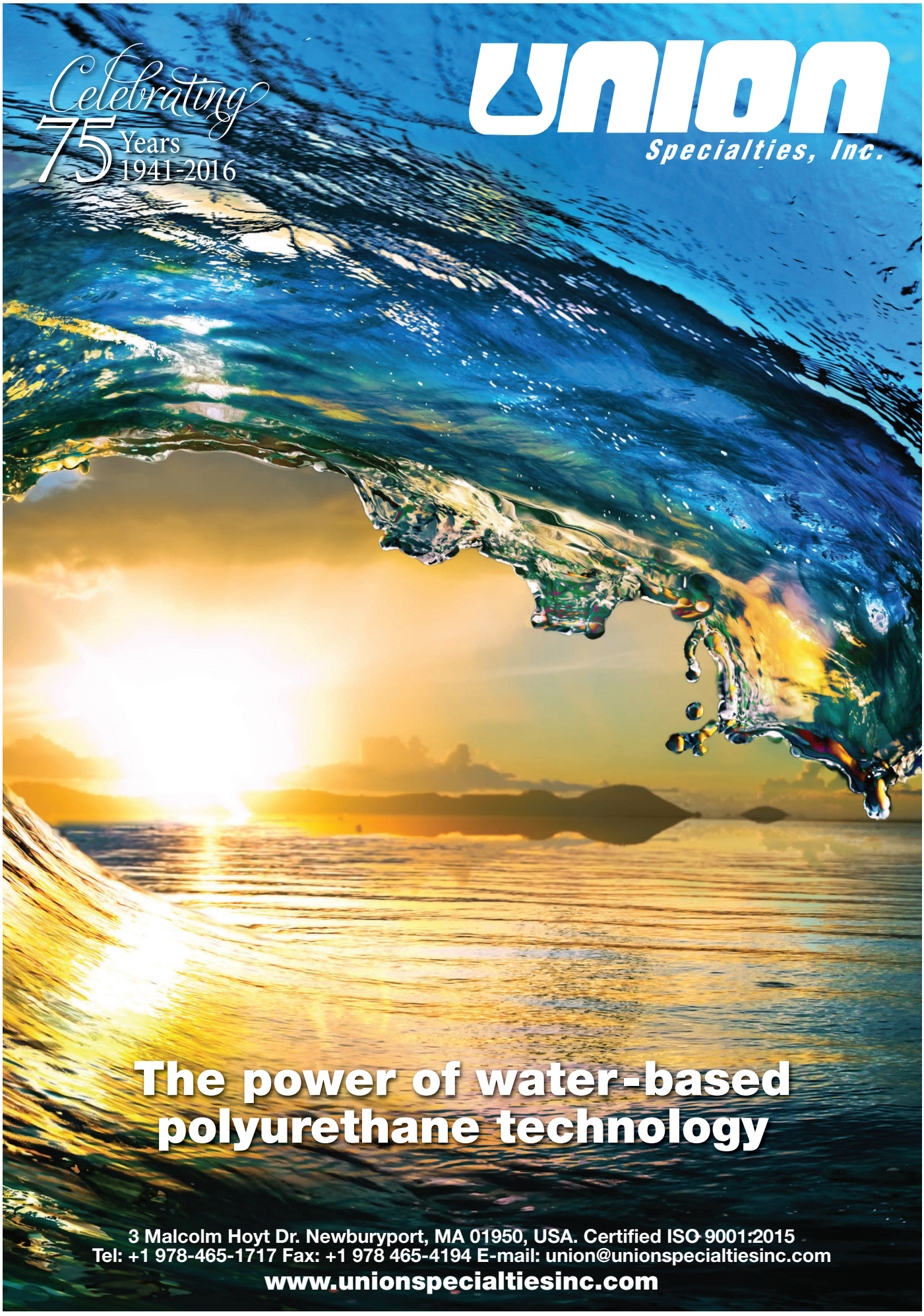
PolyMatte®

COLD Milling

Smooth Leather Milling

Erretre
Tannery Innovations

Erretre s.p.a. | Via Ferraretta,1 | Arzignano (VI) 36071 | tel. +39 0444 478312 | info@erretre.com


Celebrating
75 Years
1941-2016
UNION
Specialties, Inc.
The power of water-based polyurethane technology
3 Malcolm Hoyt Dr. Newburyport, MA 01950, USA. Certified ISO 9001:2015
Tel: +1 978-465-1717 Fax: +1 978 465-4194 E-mail: union@unionspecialtiesinc.com
www.unionspecialtiesinc.com

THE AMERICAN LEATHER CHEMISTS ASSOCIATION
1314 50th Street, Suite 103, Lubbock, Texas 79412-2940
Ph: 806-744-1798 **Fax:** 806-744-1785 **Web:** www.leatherchemists.org
Email: carol.adcock@ttu.edu

Past Issues of *Journal* Needed

If you or someone you know wants to donate past issues of the *Journal* to the Association, please contact us.

Below is a list of issues needed.

Missing Issues:

1906–1909; 1911; 1913; 1915; 1921
1937 – January and December
1939 – January, March and April
1940 – January

Extremely Low Supply:

1931 – February
1936 – January and February
1944 – Jan., Oct., Nov. and Dec.
1945 – June
1946 – January – April
1947 – January, April and August
1948 – January – March
1949 – January – June
1955 – January
1969 – March
1970 – February
1979 – July
1983 – March
1984 – July
1999 – June and July
2000 – January and February
2001 – May and June
2002 – January and March

INDEX TO ADVERTISERS

116th ALCA ANNUAL CONVENTION June 16-19, 2020 Eaglewood Resort & Spa Itasca, IL

Featuring the 61st John Arthur Wilson Memorial Lecture By Randy Johnson, President and CEO of GST AutoLeather

Tentative Schedule

Tuesday, June 16
Golf Tournament, Opening Reception and Dinner

Wednesday, June 17
John Arthur Wilson Memorial Lecture
All Day Technical Sessions, Fun Run
Reception and Dinner, Activities - Bowling, Pool, Darts and an Open Bar

Thursday, June 18
All Day Technical Sessions, Annual Business Meeting
Activities Awards Luncheon
Social Hour, ALCA Awards Banquet

Visit us at www.leatherchemists.org for full details under Annual Convention as they become available


www.CHEMTAN.com
Made with
WATERPROOF
TECHNOLOGY
CHEMTAN
Tel: (603) 772-3741 • Fax: (603) 772-0796 • www.CHEMTAN.com