Paper B1

Ozone as a Biocide in Soaking [1]

Mehmet Mete Mutlu[1], Bilge Hilal Cadirci[2], Hasan Ozgunay[1],
Arife Candas Adiguzel Zengin[1] and Ozcan Sari[1]
[1.] Department of Leather Engineering, Faculty of Engineering, Ege University, 35100, Izmir, Turkiye, Phone: 90-232-3112644, Fax:90-232-3425376
[2.] Department of Biology, Faculty of Science, Ege University, 35100, Izmir, Turkiye

The soaking process poses the most significant bacterial threat to the raw material amongst all the beamhouse processes. In soaking various bactericides are used in order to avoid possible defects, these chemicals which are lethal to bacteria may also be harmful to the environment and human health. As an alternative to these chemical based bactericides, the possible use of ozone - which is used as an antimicrobial agent in various industries - in the leather industry is investigated. The effect of ozone applications of 5, 10 and 15 minutes per hour were investigated comparing it to the effect of a sodium dimethyl dithiocarbamide based bactericide. 15 minutes of ozone application has been found to be the most effective on bacterial growth prevention.

1.Introduction

The high amount of water and the protein content of raw hides/skins makes them a very suitable nutrient environment for bacteria and thus, they are generally cured by various methods after flaying. Most of these cure methods are based on dehydration of raw hides/skins, so that the bacterial activities are limited.

The first process of leather making is soaking with the aim of removal of preserving salts, dirt, blood, unstructured proteins, proteoglycans, and rehydration of the raw hides/skins in order to prepare them for subsequent processes. Rehydrating the hides/skins allows bacteria to begin to grow very rapidly and produce enzymes.[1]

More than 80 aerobic and anaerobic microorganism species are known that can putrefy hides/skins. An important group of these species is the proteolitic bacteria that are activated in neutral or alkali pH values. This group has many members and in leather processing these are frequently observed - for example *Bacillus, Clostridium, Proteus, Esherichia, Micrococcus, Pseudomonas, Staphyloccus, Sarcina* species.[2]

The longer the soaking process, the greater is the potential for microbial growth. In fact, of all of the beamhouse processes this one poses the most significant bacterial threat to the raw material. Unless the soak water is immediately treated with an effective bactericide, the microbial population quickly

[1] This study was published in Journal of the Society of Leather Technologists and Chemists, 93 (1), 2009.

increases. It is not unusual to find millions of bacteria per millilitre of soak water within 4 to 6 hours after the soaking process begins. The rate of growth depends upon the temperature and the pH as well as the extraneous material introduced into the soak along with the hides. Most soaking processes that extend for more than 2 hours at a temperature above 16ºC will require the addition of a broad-spectrum microbicide.[3] Unless precautions are taken, bacterial activity can cause loss of hide/skin substance, low tensile strength, prominent veins, and sueded grain that decreases the value of the manufactured leather product.

Many of the bactericides previously used were based on mercury compounds, chlorinated phenols, mixtures of sulphite and acetic acid, whilst these materials are very effective they are also detrimental to the environment and are no longer used for this purpose. The substitute materials available on the market today are generally safer but may not be as effective. [1,4] Oxidation, Isothiazolone, Benzothiazole, Quaternary Ammonium Salts, Thiocyanate, Dithiocarbamate, Aldehyde, Phenol, Heavy Metals, Triazine, Phosphonium, 2-bromo-2-nitropropane and 1,3-dio (Bronopol) based commercial products are used as biocides. As many of these materials are harmful to microorganisms, it is not a surprise to expect their harm to the environment and human health. Research is being carried out both on the evaluation of the harm caused by these chemicals and to develop possible alternatives to them. In consideration of this point of view, we have researched the possible use of ozone in soaking to replace a commonly used bactericide.

Ozone (O_3) results from the rearrangement of atoms when oxygen molecules are subjected to high-voltage electric discharge. The product is a bluish gas with pungent odour and strong oxidizing properties.[5] Ozone is a water-soluble gas, which is naturally produced by the ultraviolet in sunlight. The biocide action of ozone is a result of its reaction with the double bond of fatty acids of the bacterial cell wall and membrane. Ozone application results in a change in bacterial cell permeability and leakage of cells contents into solution.[6] Another positive effect for ozone application is that ozone decomposes back to oxygen very quickly depending on temperature and relative humidity. Moreover, it does not leave any harmful by-products.[7]

In gas form ozone is harmful if it is inhaled in excess amounts. Here, as it is dissolved in water and decomposes back to oxygen very quickly it is not harmful. Additionally; at present, ozone is widely being used in the textile industry, treatment of drinking water, fish farms (disinfecting), swimming pools and spas (alternative to chemicals).

2. Materials and method

2.1 Materials

10 x 15cm samples were cut out from the croupon area of a dry salted sheep skin. A sodium dimethyl dithiocarbamide based biocide was used for comparison with the effect of ozone on bacterial growth in the soaking process.

2.2 Method

First, all samples were washed in a drum for 5 minutes in order to remove dirt and excess salts. Then the samples were put into 2lt beakers and soaked for 8 hours under the conditions given in Table 1, this is known as the logarithmic (latent) period. Each sample was stirred with a magnetic stirrer for 10 minutes in each hour to provide homogenous distribution of ozone gas and soaking bath.

In the frame of a project concerning evaluation of the pollution level and the experimental effect of ozone upon some bacterial indicators of a protected ecosystem, water samples were estimated microbiologically before and after saturation with ozone gas for 5, 10, 15, 20 and 30 min. It was found that the ozone treatment decreased the bacterial effect, considering all the bacterial indicators, by reducing their number by more than six fold after 15 minutes application.[8]

Table 1: Soaking Trials' Conditions

Trial	***Application***
Blank **(K)**	None
Biocide **(B)**	0.15% Sodium dimethyl dithiocarbamide based biocide
Ozone_5 **(O_5)**	20g/hour ozone, 5 minutes per hour
Ozone_10 **(O_{10})**	20g/hour ozone, 10 minutes per hour
Ozone_15 **(O_{15})**	20g/hour ozone, 15 minutes per hour

Water samples were taken from soaking baths at the end of every 1-hour period and bacterial populations were calculated.

2.3 Ozone production and application

Ozone gas was produced with an OGL 20 Ozone generator (Opal su ve Gida Teknolojileri Company). The OGL 20 Ozone generator uses air oxygen as input and can produce 10 or 20 gr ozone per hour by the corona-discharge principle (Figure 1).

Ozone gas was applied to the soak baths with the help of a diffuser. Ozone measurements of the water samples were done with Palintest 1000 ozonemeter (single parameter photometer) according to Palintest DPD method.

In view of the effectiveness of our tests, we are hopeful that it is feasible to employ a large capacity ozone generator in a tannery. Instead of applying the ozone with a diffuser, we think that it is better to apply it to the soak baths by a venturi system which mixes ozone and water more effectively and results in better solubility. As the second step of research, we are planning to apply it in a leather factory and to determine its effects on final leather quality.

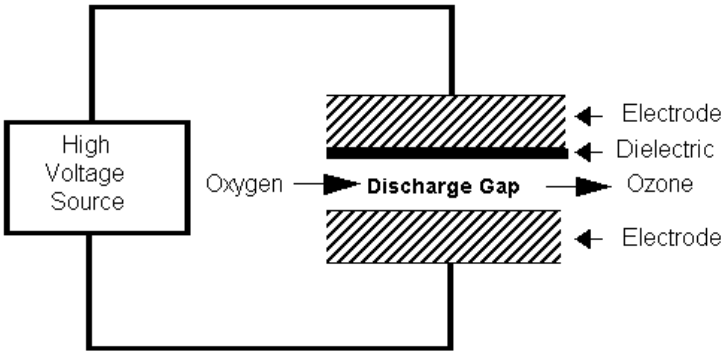

Figure 1: Ozone gas production according to Corona-discharge principle[9]

2.4 Total mezophilic bacteria determination

Total mezophilic bacteria in water samples taken hourly from soaking baths were counted on Plate Count Agar (Merck) by using the Tube dilution plate method. Colonies were counted from appropriate dilutions after 24 hours of incubation at 37ºC.

3. Results

Figure 2 illustrates the bacterial population growth graph according to count values (cfu/ml) obtained from the Blank (K) trial, which represents the soaking bath without any biocide. It is obvious that, the population reaches the highest level (6.8 x 10^6) at the end of 7th hour.

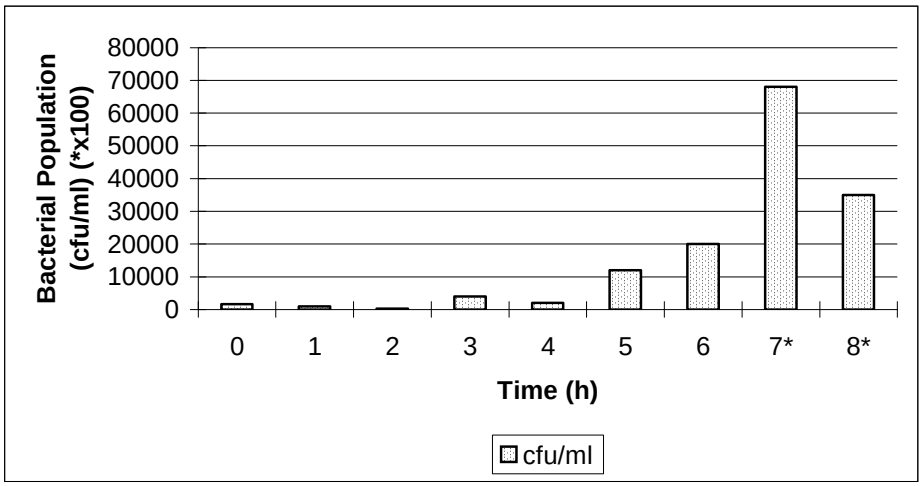

Figure 2: Bacterial growth graph of Blank trial

Colak found bacterial population in soaking baths of 1.74 x 10^4 cfu/ml at the end of 8 hours and 1.01 x 10^6 cfu/ml after 24 hours.[10] Orlita has also studied bacterial populations of various tannery effluents and detected 8.2 x 10^5 cfu/ml after 24 hours soaking.[11]

The levels of bacteria found in this work were higher than those reported elsewhere but these trials were performed in summer at temperatures about 38-40°C. Considering these conditions, 10^6 – 10^8 levels are not surprising. In one study in June 2008, we found levels of 10^7, confirmed by replication.

Bacterial growth of the B, O_5,O_{10},O_{15} trials that were expected to have a limiting effect on bacterial growth in the soaking baths are shown in Figure 3.

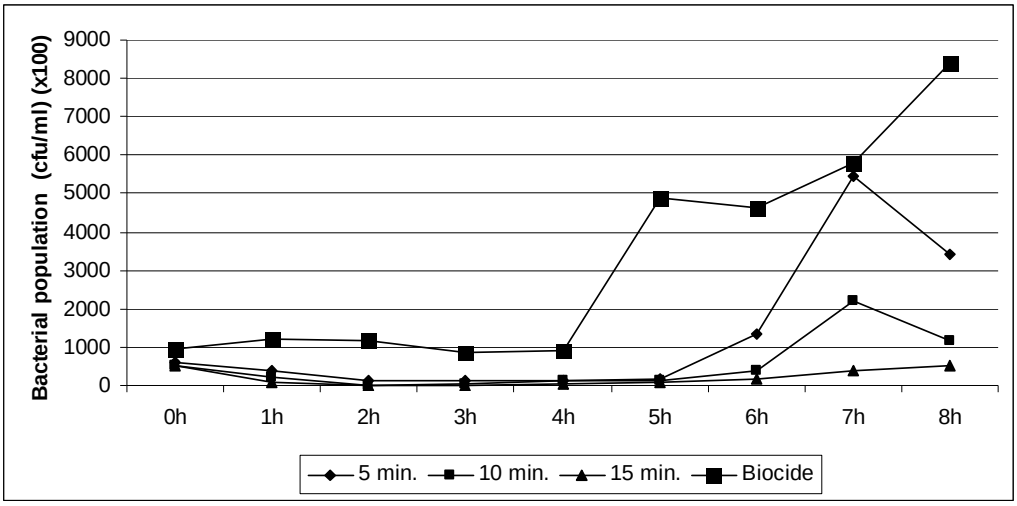

Figure 3: Bacterial population of B, O_5,O_{10},O_{15} trials

The amounts of ozone gas in the aqueous medium for the different O_5,O_{10},O_{15} trials, were measured and found to be respectively 0.33, 0.38, 0.65 mg/L.

Physicochemical factors like humidity, temperature, pH, ozone concentration levels, type of the microorganism and ozone exposure duration, determine the death rate of bacteria exposed to the ozone action.[12]

Additionally, it was thought that measuring the protein concentration in the soaking bath could be helpful to investigate whether ozone caused any damage in collagen structure that might cause a quality loss in leather-protein analysis was performed using the Bradford method[13] applied to samples taken from soaking baths at the end of the 8th hour. The amount of protein in the samples without any bactericidal treatment was 0.22 mg/ml, and was 0.01 mg/ml in the samples with bactericide application, however no protein was detected in the samples with ozone application.

4. Conclusions

When no precautions were taken against bacteria, the population reached the maximum level of $6.8x10^6$ cfu/ml - which could damage the hide/skin material - at the end of 7 hours.

No visible grain damage occurs during soaking and liming up to a total count of 10^4 bacteria per ml, 10^4- 10^6/ml represents medium infection, 10^6 upwards heavy infection (grain damage).[14]

The results of the trials with biocide or ozone application to control the bacteria population showed that;

The commercial bactericide kept the bacteria population under control for a stated time.

All the ozone trials have given results that show greater effectiveness in controlling the bacteria population than the bactericide and a relationship was observed between bacterial population decrease and the time of ozone application and thus the amount of ozone gas dissolved in the soak liquor.

15 minutes ozone application per hour was found to be the most effective in prevention of bacterial growth, however 5 and 10 minutes ozone applications kept the bacteria population level under control at a level which would not damage hide/skin.

In view of the findings, ozone can be considered as a protective agent against bacterial activity and related damage in the soaking process.

In this study, we have focused on the antibacterial effect of ozone. We checked whether there is a protein loss in skins with the Bradford method. Its results showed that there is no protein loss due to the ozone application. Regarding later processes further studies are required. As mentioned, we are planning another study at plant and industrial scale to verify ozone's possible use in soaking and to determine properties of the leathers treated with it (see *J. Amer. Leather Chem. Ass.,* 2006, **101**, on 'Studies on Ozone Based Depilation', which states ozone application did not impact on leather quality; organoleptic and strength properties were comparable).

5. References

1. Bailey, D. G., Leather Technologists Pocket Book, Preservation of Hides and Skins, 1999, SLTC, 8.
2. Karaboz, I., Deri Mikrobiyolojisi Ders Teksiri, 1994, E.U. Fen Fakültesi.
3. Didato, D., Bowen, J., Hurlow, E., Leather Technologists Pocket Book, Micorganism Control During Leather Manufacture, 1999, 339.
4. Muthusubramanian, L. and Mitra, R.B., Journal of Cleaner Production, 2006, **14**, 536.
5. Horvath M, Bilitzky L, Huttner J., Ozone, 1985, Elsevier, 350.
6. Chang, C.Y., Chin, C.Y., Lee, S.J., Huang, W.H., Yu, Y., Lion, H., et al., Ozone: Science and Engineering, 1996, **18**, 183.
7. Graham, D. M., Food Technologist, 1997, **51**, 72.
8. Voidarou, C., Tzora, A., Skoufos, I., Vassos, D., Galogiannis, G., Alexopoulos, A., and Bezirtzoglou, E., Water, Air, and Soil Pollution, 2007, **181(1-4)**, 161.
9. Angela D. Miller, William R. Grow, Leigh Ann Dees, Michael R. Mitchell, Thomas J. Manning, A history of patented methods of ozone production from 1897 to 1997, http://www.valdosta.edu/~tmanning/research/ozone/, 2008.
10. Colak, S. M., JSLTC, 2006, **90 (5)**, 193.
11. Orlita, A., Microorganisms in the Tannery, Biological Aspects of Leather Manufacture, 1969, CLRI, Madras, 1.
12. Fisher, C. W., Lee, D., Dodge, B. A., Hamman, K. M., Robbins, J. B., & Martin, S. E., Applied and Environmental Microbiology, 2000, **66(4)**, 1405.
13. Bradford, M.M., Analytical Biochemistry, 1976, **72**, 248-254.
14. http://www.schillseilacher.de/sundsdb/upload_data/en_Beamhouse_Technology_update_2006.pdf, 2008.