Destruction of Archaeal and Bacterial Communities in Leather Industry Using Electric Current

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Abstract

Hides harbor different microorganisms originating from the animal's normal flora, air in the cattle barns, feces, animal feeds, soil and contaminated water. Salt curing process is applied to hides to prevent the growth of these microorganisms. Our studies showed that microbial activities on the salted hides could not be prevented with the commonly applied salt curing process. The goal of the study is to explain species of microorganisms on the salted hides, determine the factors that prevent their inactivation and introduce the new technique to destroy these microorganisms. In our previous studies, high numbers of extremely halophilic *Archaea*, Gram positive and Gram negative *Bacteria* were isolated from the salted hides which were salt-pack cured in different countries. Most of our isolates exhibited enzymatic activities that may cause considerable decrease in the leather's quality. Despite salt saturation of these hides, bad odor, red heat and hair loss were observed nonetheless, and adhesive bacterial layers on the salted hides were detected. Our recent studies demonstrated that electric current treatment may destroy harmful bacteria in brine curing, first and main soak liquors. 0.5 A direct electric current destroyed protease and lipase producing extremely halophilic *Archaea* in the brine solution. Furthermore, 0.5 A direct electric current inactivated extremely halophilic *Archaea* in the salt samples dissolved in the brine solution. *Bacteria* in the soak liquors were inactivated with 2 A direct electrical current. Also, 1.5 A alternative electric current treatment applied together with 1.5 A direct electric current destroyed bacterial populations in the brine curing process.

Keywords: Electric current, Archaea, Bacteria, Leather Industry

Introduction

Microorganisms are highly successful organisms which are found to grow almost everywhere and affect fungi, plants, animals and human life in different ways. Although some of them are pathogenic for humans and animals, most of them serve recyclers of nutrients in nature. They are very important for carbon, nitrogen and sulfur cycles in nature. In addition to their importance in nature, a wide variety of industrial products such as antibiotics, alcoholic beverages, vinegar, yogurt, citric acid, vitamins, amino acids, steroids, and enzymes are produced by microorganisms (Madigan et al. 2012; Tortora et al. 2009; Bauman 2011).

Different species of microorganisms are usually found in soil and all animals come into contact with microorganisms found in soil because of the food chain. Therefore, whenever the animal eats grass it also ingests microorganisms from soil in low numbers. Furthermore, animals harbor different species of microorganisms as normal bacterial flora in the mouth, nose, stomach, small intestine, large intestine, skin and urethra. When the animals are alive, these microorganisms are usually harmless. After the animal is slaughtered, these microorganisms grow rapidly and damage the hide (Dahl 1956). In addition to normal flora and soil bacteria, salt used in preservation of hides likewise contaminates hide with halophilic *Bacteria* and *Archaea* and cause deterioration of hides. It was found that the salt samples, which were used in hide preservation, contained 10^2 -10^5 c.f.u./g of extremely halophilic *Archaea*. Proteolytic (10^2-10^4 c.f.u./g) and lipolytic (10^2-10^4 c.f.u./g) halophilic *Archaea* were isolated from all of the salt samples examined (Berber and Birbir 2010). Our previous studies showed that halophilic Archaeal cell counts in salt samples increased on hides. Due to high organic contents of hides, bacteria find an ideal growth media to reproduce. It was seen that the salted hide samples preserved in different countries such as Greece, England, U.S., Serbia, Bulgaria, Russia, Africa, Australia and Turkiye contained extremely halophilic *Archaea* (10^3-10^8 c.f.u./g) in higher numbers than that of salt samples (10^2 -10^5 c.f.u./g) (Berber and Birbir 2010; Aslan and Birbir 2011a). It was found that 94 % and 81 % of the hide samples had proteolytic extremely halophilic Archaea (10^2-10^6 c.f.u./g) and lipolytic extremely halophilic Archaea (10^2-10^6 c.f.u./g), respectively (Berber and Birbir 2010). Moreover, it was detected that non-halophilic *Bacteria* (10^4-10^8 c.f.u./g) was prevalent on the salted hide samples. 97 % of the hide samples had proteolytic (10^2-10^6 c.f.u./g) and lipolytic non-halophilic *Bacteria* (10^3-10^7 c.f.u./g) (Berber and Birbir 2010). Results obtained from these investigations confirmed that salted hides contained *Archaea* and *Bacteria* in high numbers. These results affirmed that salt curing method applied worldwide was not adequate to impede bacterial activities on the salted hides during storage (Berber and Birbir 2010; Aslan and Birbir 2011a).

It can be predicted that non-halophilic bacterial populations will continue to live in soak liquors. We found that soak liquors contained high number of non-halophilic *Bacteria*. Although most tanneries add antibacterial agents into the soak liquors, high bacterial cell counts were obtained from the soaked hides (10^5-10^8 c.f.u./g) and soak liquors (10^5-10^7 c.f.u./g) (Berber and Birbir 2010).

Remarkable results were obtained from salt-pack cured hides collected from different tanneries in Leather Organized Tannery Region, Tuzla-Istanbul, Turkiye. 40% of the examined hides were imported from abroad. It was found that these salted hides contained a wide variety of bacterial species belonging to Gram positive and Gram negative *Bacteria* (Aslan and Birbir 2011b; Aslan and Birbir 2012). A total of 21 different Gram-negative genera (*Acinetobacter, Aeromonas, Alcaligenes, Burkholderia, Citrobacter, Comamonas, Edwardsiella, Enterobacter, Escherichia, Hafnia, Klebsiella, Mannheimia, Pasteurella, Proteus, Pseudomonas, Salmonella, Serratia, Sphingomonas, Stenotrophomonas, Vibrio* and *Yersinia)* and 46 different Gram negative bacterial species were isolated and identified from the hide samples. The percentage of proteolytic, lipolytic and both proteolytic and lipolytic Gram-negative isolates on the hides were found as 68%, 52% and 43%, respectively (Aslan and Birbir 2011b). A total of 12 different Gram positive genera (*Aerococcus, Aneurinibacillus, Bacillus, Brevibacillus, Enterococcus, Geobacillus, Kocuira, Lactococcus, Paenibacillus, Streptococcus, Staphylococcus* and *Virgibacillus*) and 47 Gram positive

bacterial species were isolated and identified from the hides. The percentage of proteolytic, lipolytic and both proteolytic and lipolytic isolates on the hides were found as 70%, 69% and 57% (Aslan and Birbir 2012).

Like all living things, bacteria will fight to survive in adverse conditions. Microorganisms are very successful organisms on the earth in this respect. They have several resistance capacities and virulence factors, and these features explain the presence of a wide variety of bacterial flora on the salted hides and soaked hides treated with antibacterial agents.

The ability of specific adherence and invasion, hydrolytic enzymes, degradation capacity of toxic chemicals, toxins, antibiotic production, resistance against antimicrobials, salt tolerance, mutation, plasmid, porins on cell wall, efficient efflux pump systems that eject antimicrobials from the cell, flagella, fimbriae, pili, pigments, cell surface polysaccharide O antigen, cell wall, slime layer, capsule, neutralizing agents against toxic oxygen forms, endospores of bacteria may contribute to pathogenity and colonization of bacteria on the salted hides and soaked hides. (Madigan et al. 2012; Tortora et al. 2009; Bauman 2011; Balows et al. 1981). As seen, bacterial cells have a wide variety of abilities to survive in the presence of antibacterial agents and to damage their host.

What's more, virulence genes related with toxin production, adhesion, invasions, antibiotic resistance, production of bacteriocins and the other virulence factors of pathogenic bacteria may be found in transposons, plasmids or bacteriophages. These genes can be transferred among the bacteria sharing the same environment (Hacker et al.1997). Thus, bacteria living in the same environment can have similar virulence factors. Therefore, it is not easy to kill them with commonly used applications in the leather industry.

Because of their successful defense mechanisms, researchers have been obliged to develop different inactivation methods to kill bacteria. Among these treatments, electric current applications have been used to destroy a wide variety of microorganisms found in different environments.

Use of electric currents reported in the literature

The inactivation of bacteria in milk via electric current was originally proposed by Anderson and Finkelstein (1919), Beattie and Lewis (1925), and Prescott (1927). Researchers found that electric current might be used in sterilization of milk. It was stated that this inactivation method was fairly effective to inhibit bacterial growth in milk (Anderson and Finkelstein 1919; Beattie and Lewis 1925; Prescott 1927). Afterwards, the other researchers used electric current to kill bacteria found in different environment such as salt solutions (Pareilleux and Sicard 1970), on human skin (Bolton et al.1980), in synthetic urine (Davis et al.1991), on chicken legs (Slavik et al. 1991), on the surface of catheters (Liu et al.1997), in activated sludge and biofilms (Li et al. 2001), in seawater (Park et al. 2003), in river water and sea water (Birbir et al. 2009), on beef surfaces (Mahapatra et al. 2011). Researchers used different levels of electric currents to kill different species of bacteria such as *Escherichia coli*, *Pseudomonas aeruginosa* (Tokuda et al. 1995), *Escherichia coli* and *Listeria monocytogenes* (Venkitanarayanan et al. 1999), sulfur-oxidizing bacteria, *Thiobacillus ferrooxidans*, *acidophilic heterotroph acidiphilium* (Jackman et al. 1999), *Vibrio parahaemolyticus* (Park et al. 2003) and nitrifying bacteria (Li et al. 2001).

Following these results, our recent studies examined the efficiency of electric current for killing *Archaea* and *Bacteria* isolated from salts and salted hides and soaked hides. Firstly, inactivation effect of direct electric current against extremely halophilic *Archaea* isolated from salt samples was examined. Both lipase and lipase-plus-protease producing extremely halophilic *Archaea* of 10^7 c.f.u/mL in presence of organic substances were killed in 20 min via 0.5 A low-level direct electric current. While a mixed culture of extremely halophilic *Archaea* was inactivated within 10 min, protease producing extremely halophilic *Archaea* were inactivated within 5 min (Birbir and Birbir 2006). Afterwards, we evaluated that whether electric current can be used for sterilization of brine solution which will be used in brine curing of hides. It was found that low level electric current was fairly effective to kill extremely halophilic *Archaea* in salt samples. In that study, the extremely halophilic archaeal populations (10^2-10^5 c.f.u./g) in all 40 salt samples dissolved in the brine solution were inactivated by 0.5 A direct electric current within 1 min. However, long exposure time (15 min) of electric treatment was necessary to inactivate extremely halophilic *Archaea* (10^5 c.f.u./g) in 5 salt samples dissolved in the presence of organic substances (Birbir et al. 2008a)

Later, we examined the bactericidal effect of direct electric current on bacteria in hide-soak liquors which are discharged into the environment without any treatment. Therefore, we concentrated on the effect of 2 A direct electric current on bacterial populations in the first and main hide-soak liquors. A total of 14 soak liquors containing the first hide-soak (Sample 1-7) and main hide-soak (Samples 8-14) liquors were collected from different tanneries in the Tuzla-Istanbul Leather Organized Tannery Region in Turkiye. In that experiment, gram-negative (five samples) and halotolerant bacteria (seven samples) were isolated from both first and main hide-soak liquors. Although the mean value of the mesophilic bacterial numbers in the first hide-soak liquors was usually 10^5 c.f.u./mL, the mean value of mesophilic bacterial numbers in the main hide-soak liquors was 10^6 c.f.u./mL. Bacteria in all of the first hide-soak liquors and three main soak liquors were inactivated after 30 min via 2 A direct electric current. Bacteria in four main hide-soak liquors were inactivated after 50 min. The bacterial populations in all of the soak liquors steadily lost their viability during the electrical treatment. The most remarkable result is that there was a correlation between voltage level and numbers of bacterial cell in the electrolysis cell. Bacterial population in the soak liquors decreased proportionally to voltage level until the entire bacterial population was killed. After the bacterial populations in the soak liquors were completely destroyed by electric treatment, voltage levels started to increase. In the hide industry, this important clue might be used to predict extermination time of the bacterial population in soak liquors (Birbir et al. 2008b).

The effect of AC and DC treatments for inactivation of commonly found Gram negative bacterial isolates (*Enterobacter cloacae, Pseudomonas luteola* and *Vibrio fluvialis*) on salt-pack cured hides and possible use of this treatment system in the leather industry were examined in our other research. Experiments were carried out with Gram-negative bacterial isolates that produce protease, Gram-negative bacterial isolates that produce protease and lipase and with a mixed culture of these bacteria in brine containing 25% NaCl. *Enterobacter cloacae, Pseudomonas luteola* and *Vibrio fluvialis* in the brine solutions containing 25% NaCl were completely inactivated in 15 minutes *via* 1.5A AC treatment. In that investigation, the mixed population of test bacteria in the brine solution containing 25% NaCl was completely inactivated after treatment with 1.5A AC for 15 minutes followed by 1.5A DC for 1 minute. Our results showed that bacterial cells which were damaged by AC in the brine curing process

may be inactivated further with DC in a short time without causing a high temperature increase during the treatment (Birbir et al. 2013).

Effect of electric current on microorganisms

Electric currents may affect the orientation of membrane lipids and consequently cell viability. It has been proposed that bacterial inactivation may be due to oxidation of vital cellular constituents during exposure to electric current. Furthermore, hydrogen peroxide, ozone, free chlorine and chlorine dioxide which are produced by electrodes during electric current treatment may kill bacteria. It has been observed that direct electric current application to microorganisms in seawater caused considerable structural damage at cellular level and irreversible cell membrane rupture in different parts of the cell membrane, leading to the apparent leakage of intracellular contents. When electrochemically generated oxidants are present, these pores may allow the oxidants free access to the interior of the cell, aiding the inactivation process. It has been proposed that electric current causes bacterial cell membrane damage such as growth of the membrane, surface shape fluctuations and molecular rearrangements. This damage leads to a membrane discontinuity and pore expansion which can result in an irreversible membrane breakdown (Davis et al. 1994; Park et al. 2003; Liu et al. 1997; Dress et al. 2003; Chang et al. 2009).

Conclusion

Electric current applications were found fairly effective to kill *Archaea* and *Bacteria* isolated from salt, salted hides and soak liquors. Our experimental results showed that electric current applications kill both archaeal and bacterial species. It does not form mutant or resistant strains, it is cheap, easy and simple, its diffusion rate to aggregated microorganisms or microorganisms attached to other cellular debris is higher than antibacterial agents, and it kills the *Bacteria* and *Archaea* in a short treatment time. Therefore, we suggest applying this system in hide industry to inactivate extremely halophilic *Archaea* in salt and *Bacteria* in soak liquors.

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