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Annihilation of Extremely Halophilic Archaea in Hide Preservation Salt Using Alternating Electric Current

Electrochemical disinfection of hide brine liquors for use in the leather industry

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Salt contains extremely halophilic archaea and these microorganisms degrade leather quality. The aim of this study is to find an effective treatment system to kill these microorganisms in salt used in hide brine curing. Ten salt samples were obtained from Tuz Lake, Turkey, and the total cell counts of extremely halophilic archaea, proteolytic and lipolytic extremely halophilic archaea were determined. Two sets of experiments were designed to detect the inactivation impact of alternating electric current on extremely halophilic archaea. In the first experiment, 2 A alternating electric current was applied for 25 min to the salt samples dissolved in liquid medium. In the second experiment, 2 A alternating electric current was applied for 25 min to the isolates of proteolytic extremely halophilic archaea, lipolytic extremely halophilic archaea, both proteolytic and lipolytic extremely halophilic archaea, and a mixed culture of these isolates. The extremely halophilic archaea in salt (10^2–10^4 colony forming units (CFU) g^{-1}) was annihilated in 1 min *via* alternating electric current and a 5 min treatment with the current was enough to destroy extremely halophilic archaeal isolates (10^6 CFU ml^{-1}) obtained from salt samples. This electric treatment was found fairly effective to kill proteolytic and lipolytic extremely halophilic archaea in salt used for preservation of hide.

1. Introduction

The leather industry is an important sector in the world economy. Almost 23 billion square feet of leather is produced annually (1, 2) and the total value of this is estimated to be more than US$100 billion (3). Most of the global earnings are accrued from footwear sales. This sector represents 60% of the industry's total income, with annual production of 13 million pairs of shoes (4, 5). Because many consumers can perceive when leather products are manufactured from an inferior hide grade, producers are perforce obliged to dispose of damaged hides. Ignorance of effective preservation techniques therefore causes important economic losses in the industry.

Studies have shown that hides contain extensive populations of Gram-positive and Gram-negative bacteria which may be either resident or transient bacteria. As soon as the animal is slaughtered, these bacteria can grow on the raw hides and degrade the hide quality (6–8). A mixture of salt and boric acid is commonly used to prevent the growth of these microorganisms and preserve the hides. If hides are preserved with only crude solar salt, they can be contaminated by different species of extremely halophilic archaea (9–12). These microorganisms are easily detected on hides as they are revealed by red to orange pigments. Extremely halophilic archaea produce red, orange and pink coloured colonies because of the presence of C_{50} carotenoids (9–12). Red discolorations on the salted hides, caused by microorganisms, have been scrutinised by researchers since 1929; even at that early date, proteolytic activity of halophilic microorganisms was recognised (13). In addition, occurrence of red colouration produced by extreme halophiles has been noted in the Great Salt Lake (14), Dead Sea (15), Lake Magadi (16) and Tuz Lake (11). Salt lakes and solar saltern crystalliser ponds contain 10^7–10^8 CFU of extremely halophilic archaea per ml (9). In previous studies, salt samples which were obtained from Tuz Lake in Turkey contained 10^4–10^7 CFU g^{-1} extremely halophilic archaea (11). In another study, the viable cell number of extremely halophilic archaea in water samples collected from Tuz Lake was found to be 1.38×10^7 CFU ml^{-1} (17).

Salt collected from Tuz Lake, located in Central Anatolia, Turkey, is used to preserve hides in the Turkish leather industry. If the salt obtained from Tuz Lake is directly used in hide preservation, it may contaminate hides with halophiles. During long storage or overseas transportation of the salted hides, viable cell numbers of extremely halophilic archaea on the hides can multiply excessively. In an earlier study, extremely halophilic archaea were observed in considerably high numbers (10^3–10^8 CFU g^{-1}) on 36 salt-pack cured hides. Extremely halophilic archaeal counts were fairly high in 72% of the hides (10^5–10^7 CFU g^{-1}) as a consequence of inadequate salt-pack curing method. 94% and 81% of the samples contained proteolytic extremely halophilic archaea (10^2–10^6 CFU g^{-1}) and lipolytic extremely halophilic archaea (10^2–10^6 CFU g^{-1}), respectively (18). These findings were consistent with previous studies' reports of the extremely halophilic archaeal counts (10^5–10^8 CFU g^{-1}) on the brine cured hides (19, 20).

The destructive effect of extremely halophilic archaea on salted hides has been observed. It was detected that proteolytic extremely halophilic archaea, originating in unprocessed solar salt, digested the grain surface of the hide and caused a complete disruption of collagen fibers (21). Furthermore, it was noted that proteolytic and lipolytic extremely halophilic archaea produced sponge-like vesicles within the hide and light stains on the suede surface of finished double-face leathers (22, 23). Therefore, inadequately preserved hides and skins may adversely affect final leather quality.

Due to these destructive effects of extremely halophilic archaea on the salted hides, an effective, easy and inexpensive method should be applied to eradicate these archaea in salt. Thus, using sterile salt in hide preservation will increase leather quality and prevent substantial economical losses in the leather industry.

1.1 Use of Electric Current

Electric current application is one of these methods. This treatment system has been used by several researchers to inactivate different types of microorganisms found in synthetic urine (24), water (25), fresh orange juice (26), activated sludge and biofilms (27), effluent seawater (28), soak liquors (29), brine solution (30, 31), on beef surfaces (32) and chicken legs (33). In those experiments, scientists usually used direct electric current to eliminate diverse bacterial populations.

In addition to direct electric current, alternating electric current was also used to inactivate bacteria in phosphate buffer solution (34), seawater (35) and natural waters (36). Researchers mentioned that alternating electric current treatment has advantages over direct electric current (35). The investigators found that application of 1 A direct electric current or 1 A alternating electric current for 100 ms were sufficient to kill *Vibrio parahaemolyticus* in seawater. They concluded that 1 A alternating electric current application at 5 Hz generated less chlorine concentration (<5 mg l^{-1}) in seawater than when the seawater was treated with direct electric current (approximately 35 mg l^{-1}). It was revealed that this reduced production of chlorine was due to the bidirectional flow of alternating current, resulting in less electrolysis of the test medium. It was also emphasised that chlorine gas produced in direct electric current treatment may cause serious health problems in humans (35). To overcome this adverse effect of chlorine gas, the researchers suggested using alternating electric current to

kill bacteria in natural waters (36) and industrial applications (35).

The mechanism of bacterial inactivation by alternating electric current has been explained as a byproduct of chlorine (36, 37) and hydrogen peroxide (34, 35, 38) produced during electrolysis. Previous experiments demonstrated that electric field and current caused cell membrane damage in the form of pores that opened on the membrane following electrical treatment (39, 40). Chloride and hydrogen peroxide, produced during electrolysis, may enter easily through these pores, thus hastening the inactivation process (39, 40). In addition, researchers observed that application of alternating electric current on *Escherichia coli* cells damaged their cell membranes, thereby causing the leakage of intracellular components, DNA-related materials and ninhydrin-positive materials from the cell (41). Moreover, alternating electric current affected *E. coli* cells by increasing the negative charge at the surface and decreasing their respiratory rate (42). Furthermore, the amount of phosphatidylethanolamine in the cell membrane of *E. coli* was reduced by electric current treatment (43).

Although studies indicate that direct electric current can annihilate different species of microorganisms in diverse industries (24–33), the inactivation effect of alternating electric current on extremely halophilic archaea in hide curing salt has not yet been evaluated. The alternating current disinfection method was used in the present study precisely because it was proven to release only a low chlorine concentration (35). Therefore, the experiments were designed to evaluate the following: the inactivation impact of 2 A alternating electric current, which produces less chlorine than direct current treatment (35), on extremely halophilic archaea in salt; the inactivation effect of 2 A alternating electric current on the isolates of proteolytic extremely halophilic archaea, lipolytic extremely halophilic archaea, both proteolytic and lipolytic extremely halophilic archaea and a mixed culture of these isolates. The total extremely halophilic archaeal counts, total proteolytic extremely halophilic archaeal counts and total lipolytic extremely halophilic archaeal counts in salt samples were also determined.

2. Experimental procedures

2.1 Determination of the Total Counts of Extremely Halophilic Archaea

Ten salt samples were collected from Tuz Lake in Turkey. A total of 20 g of salt sample was separately placed into an electrolysis cell containing a final volume of 200 ml of 25% NaCl solution in water. Then, this mixture was placed into a shaker incubator (100 rpm) for 4 h at room temperature. A total of 100 µl of the test medium was removed from each electrolysis cell before commencing the experiments. This was diluted to 10-fold (10^{-1}) dilution, 100-fold (10^{-2}) dilution and 1000-fold (10^{-3}) dilution with sterile 25% NaCl solution. The viable extremely halophilic archaeal cell numbers in these samples were determined by the plate count method. To detect the total extremely halophilic, proteolytic and lipolytic extremely halophilic archaeal cell numbers in the salt samples, the direct and the diluted solutions were spread over complex agar medium, gelatin agar and Tween® 80 agar media, respectively, and incubated at 39ºC for 30 days. After the incubation period, the colonies on the complex agar surface were counted. To determine protease positive isolates, the colonies on the gelatin agar medium were flooded with Frazier solution. Then, the colonies with clear zones were considered as protease positive, and these colonies were counted (44, 45). The colonies with opaque zones on Tween® 80 agar were considered as lipase positive and were counted (44). Later, viable cell numbers of these archaeal isolates in 1 ml were calculated. Protease and lipase positive colonies with different colours and morphology were selected and streaked out several times to obtain pure cultures on complex agar medium by the streak plate technique.

2.2 The Electrochemical Disinfection System

An electrochemical cell was constructed of a glass beaker containing two internally attached platinum wire electrodes (1 mm in diameter, 80 mm long) submerged in 25% NaCl solution. The distance separating the two electrodes was 40 mm. These electrodes were attached to a regulated alternating current source (Ruhstrat GmbH, Germany) (input = 220 V, frequency = 50 Hz, power = 2250 VA), which had an automatic variable output voltage range of 0–220 V and user-selectable current range of 0–9 A (35, 46).

In the present study, 2 A alternating electric current treatment was applied to two experimental sets. In the first experimental set, consisting of ten tests, 2 A alternating electric current was applied to salt samples mixed separately into 25% NaCl solutions. In the second experimental set, consisting of four tests, 2 A alternating electric current was applied to a mixed culture of proteolytic extremely halophilic archaea, a mixed culture of lipolytic extremely halophilic archaea,

a mixed culture of both proteolytic and lipolytic extremely halophilic archaea, plus a mixed culture of these isolates added to 25% NaCl solutions.

2.3 Inactivation of Extremely Halophilic Archaea

As explained above, for the first experimental set, an electrolysis cell containing 200 ml of liquid test medium, 25% NaCl and separate salt samples was used in this experiment. 2 A alternating electric current was applied to the electrolysis cell for 25 min. Aliquots of 100 µl were removed from this medium at intervals of 1, 5, 10, 15, 20 and 25 min during the alternating current treatment. Then, the aliquots were diluted to 10-fold (10^{-1}) dilution, 100-fold (10^{-2}) dilution and 1000-fold (10^{-3}) dilution in sterile physiological saline solution. 100 µl of the direct and diluted solutions were spread over complex agar medium and incubated at 39ºC for 30 days. Then, the colonies on the agar surface were counted. The detection limit of this experiment was 10 CFU ml^{-1}.

2.4 Inactivation of Proteolytic and Lipolytic Extremely Halophilic Archaea

For the second experimental set, protease and lipase positive extremely halophilic archaea were isolated from the salt samples obtained from Tuz Lake. Two isolates of protease positive (TLPI1 and TLPI2), two isolates of lipase positive (TLLI1 and TLLI2), two isolates of both protease and lipase positive (TLPLI1 and TLPLI2) and a mixed culture of these extremely halophilic isolates (TLPI1, TLPI2, TLLI1, TLLI2, TLPLI1 and TLPLI2) were selected as test isolates to use in alternating electric current treatment experiments. Each of these isolates was cultivated for 15 days at 39ºC in a shaker incubator (Edmund Bühler, Germany) at 100 rpm in a liquid complex medium. After the incubation period, each of these extremely halophilic archaeal isolates was diluted in 25% NaCl solution to a final population density of 10^7 CFU ml^{-1}. Next, the aforementioned isolate groups were prepared. 20 ml of protease positive (TLPI1 and TLPI2), lipase positive (TLLI1 and TLLI2), both protease and lipase positive (TLPLI1 and TLPLI2) and the mixed culture (TLPI1, TLPI2, TLLI1, TLLI2, TLPLI1 and TLPLI2) were separately placed into an electrolysis cell containing 180 ml of 25% NaCl solution. Later, 2 A alternating electric current was applied to each electrolysis cell for 25 min. A 100 µl quantity of the test medium was removed from the electrolysis cell at intervals of 1, 5, 10, 15, 20 and 25 min. Each of the test media was plated onto complex agar medium both directly and after serial dilutions, then incubated for 30 days at 39ºC. After the incubation period, colonies on the agar surface were counted and CFU of extremely halophilic archaea in 1 ml was calculated. The detection limit of this experiment was 10 CFU ml^{-1}.

The $\log_{10}$ reduction factor (RF) for each treatment time was calculated according to the following formula:

$$RF = \log_{10} n_b - \log_{10} n_a$$

where n_b is the initial number of viable cells (CFU ml^{-1}) in the inoculum of the first experiment (salt samples dissolved in 200 ml of liquid medium containing 25% NaCl) or in the inoculum of the second experiment (test isolates found in 200 ml of liquid medium containing 25% NaCl); and n_a is the number of viable cells (CFU ml^{-1}) in the inoculum (salt samples or test isolates) after treatment with 2 A alternating electric current.

The pH and temperature of all samples were measured at each of the aforementioned intervals using a pH meter (Professional Meter PP-50, Sartorius AG, Germany). The temperatures of all test media were adjusted to 25ºC before application of alternating electric current treatment.

3. Results and Discussion

Extremely halophilic archaea, proteolytic and lipolytic extremely halophilic archaea were detected in all salt samples (**Table I**). Although salt samples mostly contained between 10^3 and 10^4 CFU of extremely halophilic archaea per gram, proteolytic and lipolytic extremely halophilic archaeal numbers were between 10^1 and 10^3 CFU g^{-1} in salt samples (**Table I**). These findings were consistent with previous experiments (18, 30). In those studies, 80 salt samples used in hide preservation were examined for extremely halophilic archaea. It was found that all salt samples contained 10^2–10^5 CFU g^{-1} of extremely halophilic archaea (18, 30). Moreover, all salt samples contained proteolytic and lipolytic extremely halophilic archaea (10^2–10^4 CFU g^{-1}) (18, 30).

In this study, two sets of experiments were conducted to examine the inactivation effect of alternating electric current on extremely halophilic archaea. In the first experiment, extremely halophilic archaea, proteolytic and lipolytic extremely halophilic archaea in salt samples were killed in 1 min *via* 2 A alternating electric current treatment. There was a sharp decrease in the

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Table I Numbers, Log_{10} Values and Reduction Factors of Extremely Halophilic Archaea, Proteolytic and Lipolytic Extremely Halophilic Archaea in Salt Samples Dissolved Separately in the Brine Solutions Treated by 2 A Alternating Electric Current within 1 min

Salt sample	Exposure time, min	Extremely halophilic archaea			Proteolytic extremely halophilic archaea			Lipolytic extremely halophilic archaea		
		Number, CFU ml^{-1}	Log_{10} values	Reduction factor	Number, CFU ml^{-1}	Log_{10} values	Reduction factor	Number, CFU ml^{-1}	Log_{10} values	Reduction factor
1	0	2.8×10^4	4.4	–	1.9×10^2	2.3	–	3.4×10^2	2.5	–
	1	<10	<1	>3.4	<10	<1	>1.3	<10	<1	>1.5
2	0	4.1×10^4	4.6	–	6.8×10^3	3.8	–	9.0×10^3	3.9	–
	1	<10	<1	>3.6	<10	<1	>2.8	<10	<1	>2.9
3	0	1.4×10^4	4.1	–	1.3×10^3	3.1	–	1.9×10^3	3.2	–
	1	<10	<1	>3.1	<10	<1	>2.1	<10	<1	>2.2
4	0	1.4×10^3	3.1	–	1.0×10^2	2.0	–	4.0×10^2	2.6	–
	1	<10	<1	>2.1	<10	<1	>1.0	<10	<1	>1.6
5	0	2.0×10^3	2.3	–	1.6×10^2	2.2	–	9.0×10^2	2.9	–
	1	<10	<1	>1.3	<10	<1	>1.2	<10	<1	>1.9
6	0	3.4×10^2	2.5	–	1.0×10^1	1.0	–	2.0×10^1	1.3	–
	1	<10	<1	>1.5	<10	<1	–[a]	<10	<1	>0.3
7	0	9.7×10^3	3.9	–	3.4×10^2	2.5	–	6.5×10^2	2.8	–
	1	<10	<1	>2.9	<10	<1	>1.5	<10	<1	>1.8
8	0	1.2×10^4	4.0	–	1.1×10^3	3.0	–	2.8×10^3	3.4	–
	1	<10	<1	>3.0	<10	<1	>2.0	<10	<1	>2.4
9	0	8.1×10^3	3.9	–	4.0×10^1	1.6	–	1.1×10^2	2.0	–
	1	<10	<1	>2.9	<10	<1	>0.6	<10	<1	>1.0
10	0	8.7×10^3	3.9	–	1.0×10^1	1.0	–	1.0×10^2	2.0	–
	1	<10	<1	>2.9	<10	<1	–[a]	<10	<1	>1.0

[a]The number was not detected because it was under the detection limit

numbers of extremely halophilic archaea, proteolytic and lipolytic extremely halophilic archaea in the salt samples after 1 min of 2 A alternating electric current treatment (**Table I**).

Temperature values of the test media did not change when the extremely halophilic archaea in the salt samples were killed. The temperature of all test media containing salt samples was 25ºC before the experiment and remained the same after 1 min of electric treatment. At the end of experiment (after 25 min), the temperature of these media reached 35ºC. The pH of the test media did not change during the experiment. Voltage levels of the test media containing the salt samples varied according to the samples and the duration of electric current treatment. However, all voltage levels decreased at the end of these experiments (**Table II**).

The inactivation effect of alternating electric current on Gram-negative bacteria has been examined in previous studies (34–36, 47, 48). *E. coli* cells in the test medium containing nutrient broth, peptone and NaCl were killed by 110 mA alternating electric current in 10 s (36). *E. coli* cells suspended in a phosphate buffer solution were also killed by 300 mA cm^{-2} alternating electric current (34). *V. parahaemolyticus* in effluent seawater was killed by 3 A alternating electric current treatment in 30 ms (35). In an earlier study, both *E. coli* ATCC 25922 (resistant to ampicillin (10 µg ml^{-1}) and amoxicillin-clavulanic acid (30 µg ml^{-1})) and fecal *E. coli* MAAG 1405 (resistant to ampicillin (10 µg ml^{-1}) and amoxicillin-clavulanic acid (30 µg ml^{-1}), imipenem (10 µg ml^{-1}), cefuroxime (30 µg ml^{-1}), cefotaxime (30 µg ml^{-1}) were killed within 1–3 min in media containing marine waters (1 A), 5–10 min in Ayamama River water (1 A), 5 min in Sarisu River water (0.5 A) and 20–35 min in lauryl sulfate broth (1 A) by applying alternating electric current. Log_{10} reduction factors of *E. coli* ATCC 25922 and fecal *E. coli* MAAG 1405 were between 3.47 and 3.99 in the different water samples when these bacteria were completely inactivated. The pH of all water samples was between 6 and 7 and remained constant during alternating current treatment (47). Furthermore, in another study 1.5 A alternating electric current was used to kill *Enterobacter cloacae, Pseudomonas luteola* and *Vibrio fluvialis*, as well as a mixed population of these Gram-negative bacteria isolated from hides. 15 min exposure to 1.5 A alternating electric current inactivated *Enterobacter cloacae, Pseudomonas luteola* and *Vibrio fluvialis* in the brine solution containing 25% NaCl. Moreover, exposure to 1.5 A alternating electric current for 15 min followed by 1.5 A direct current for 1 min inactivated the mixed population of these bacteria in the brine solution. The maximum temperature rise was 6ºC (48).

In the second experiment, the mixed culture of protease positive isolates (TLPI1 and TLPI2), mixed culture of lipase positive isolates (TLLI1 and TLLI2), mixed culture of both proteolytic and lipolytic isolates (TLPLI1 and TLPLI2) and mixed culture of all test isolates (TLPI1, TLPI2, TLLI1, TLLI2, TLPLI1 and TLPLI2) were prepared as four different treatment groups. In our previous study we detected proteolytic and lipolytic extremely halophilic archaea between 10^2–10^6 CFU g^{-1} in 36 salted hides (18). Hence, high populations of proteolytic and lipolytic extremely halophilic archaeal isolates (10^6 CFU ml^{-1}) were used to examine the inactivation effect of alternating electric current. 2 A alternating electric current was applied to the mixed cultures of these isolates in the test media containing 25% NaCl for 1, 5, 10, 15, 20 and 25 minutes.

As indicated in **Table III**, the archaeal cell counts in all test media also decreased during the electric treatments. The mixed culture of protease positive isolates dramatically decreased during 1 min of electric treatment. Likewise, lipase positive isolates, both proteolytic and lipolytic isolates, and the mixed culture of all isolates also decreased sharply. After 1 min alternating electric treatment, the numbers of protease positive isolates, the numbers of lipase positive isolates, the numbers of both proteolytic and lipolytic isolates, and mixed culture of all isolates decreased 1.0 log_{10}, 3.5 log_{10}, 2.1 log_{10}, and 3.2 log_{10}, respectively. In this experiment, within 5 min of 2 A alternating electric current application, all archaeal cells were annihilated in each treatment (**Table III**).

In the second experiment set, temperature values of the test media increased slightly when the test isolates were inactivated. All voltage levels decreased slightly when the test isolates were inactivated. At the end of the experiment, temperature values and voltage levels of the test media were measured respectively at 35ºC and between 4.6–5.1 V (**Table III**).

4. Recommendations

The authors of this study highly recommend this easy, effective, economical, fast, broad-spectrum activity system to prevent archaeal damage on brine cured hides. Although there are a few studies which explain the mechanism of bacterial inactivation by alternating

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Table II Values of Temperature, Voltage and pH in the Liquid Test Media Containing Salt Samples Treated by 2 A Alternating Electric Current Over 25 min

Salt sample	1			2			3			4			5		
Exposure time, min	°C[b]	V[c]	pH	°C	V	pH	°C	V	pH	°C	V	pH	°C	V	pH
0	25	–	7.57	25	–	7.73	25	–	7.52	25	–	7.09	25	–	8.03
1	25	4.9	7.57	25	5.0	7.73	25	5.0	7.52	25	7.0	7.09	25	7.0	8.03
5	27	4.9	7.57	27	4.9	7.73	27	4.8	7.52	27	6.9	7.09	27	6.9	8.03
10	29	4.8	7.57	29	4.8	7.73	29	4.7	7.52	29	6.8	7.09	29	6.8	8.03
15	31	4.7	7.57	31	4.7	7.73	31	4.6	7.52	31	6.8	7.09	31	6.7	8.03
20	33	4.6	7.57	33	4.6	7.73	33	4.6	7.52	33	6.6	7.09	33	6.6	8.03
25	35	4.6	7.57	35	4.5	7.73	35	4.5	7.52	35	6.1	7.09	35	6.4	8.03

[b]Temperature, [c]Voltage

Table II Continued

Salt sample	6			7			8			9			10		
Exposure time, min	°C	V	pH	°C	V	pH	°C	V	pH	°C	V	pH	°C	V	pH
0	25	–	7.35	25	–	7.74	25	–	8.24	25	–	7.44	25	–	7.40
1	25	7.7	7.35	25	7.6	7.74	25	7.5	8.24	25	5.8	7.44	25	5.8	7.40
5	27	7.5	7.35	27	7.4	7.74	27	7.3	8.24	27	5.7	7.44	27	5.7	7.40
10	29	7.3	7.35	29	7.2	7.74	29	7.2	8.24	29	5.6	7.44	29	5.5	7.40
15	31	7.0	7.35	31	7.0	7.74	31	7.0	8.24	31	5.5	7.44	31	5.2	7.40
20	33	6.8	7.35	33	6.8	7.74	33	6.8	8.24	33	5.2	7.44	33	5.1	7.40
25	35	6.6	7.35	35	6.6	7.74	35	6.7	8.24	35	5.1	7.44	35	5.0	7.40

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Table III Values of Temperature, Voltage and pH, Total Counts, Log_{10} Values and Reduction Factors of the Test Isolates in the Brine Media Treated by 2 A Alternating Electric Current Over 25 min

Exposure time, min	Mixed culture of proteolytic extremely halophilic isolates (TLPI1 and TLPI2)						Mixed culture of extremely halophilic lipolytic isolates (TLLI1 and TLLI2)					
	°C	V	pH	Number, CFU ml^{-1}	Log_{10} values	Reduction factor	°C	V	pH	Number, CFU ml^{-1}	Log_{10} values	Reduction factor
0	25	–	7.0	6.3×10^6	6.8	–	25	–	7.0	5.9×10^6	6.8	–
1	25	5.6	7.0	1.0×10^1	1	5.8	25	5.8	7.0	3.5×10^3	3.5	3.3
5	27	5.3	7.0	<10	<1	>5.8	27	5.7	7.0	<10	<1	>5.8
10	29	5.1	7.0	<10	<1	>5.8	29	5.4	7.0	<10	<10	>5.8
15	31	4.9	7.0	<10	<1	>5.8	31	5.2	7.0	<10	<10	>5.8
20	33	4.7	7.0	<10	<1	>5.8	33	5.1	7.0	<10	<10	>5.8
25	35	4.6	7.0	<10	<1	>5.8	35	5.0	7.0	<10	<10	>5.8

Table III Continued

Exposure time, min	Mixed culture of extremely halophilic both proteolytic and lipolytic isolates (TLPLI1 and TLPLI2)						Mixed culture of all extremely halophilic archaeal isolates (TLPI1, TLPI2, TLLI1, TLLI2, TLPLI1 and TLPLI2)					
	°C	V	pH	Number, CFU ml^{-1}	Log_{10} values	Reduction factor	°C	V	pH	Number, CFU ml^{-1}	Log_{10} values	Reduction factor
0	25	–	7.0	5.7×10^6	6.7	–	25	–	7.0	4.9×10^6	6.7	–
1	25	5.9	7.0	1.2×10^2	2.1	4.6	25	5.7	7.0	1.6×10^3	3.2	3.5
5	27	5.8	7.0	<10	<1	>5.7	27	5.6	7.0	<10	<1	>5.7
10	29	5.5	7.0	<10	<1	>5.7	29	5.4	7.0	<10	<1	>5.7
15	31	5.3	7.0	<10	<1	>5.7	31	5.2	7.0	<10	<1	>5.7
20	33	5.2	7.0	<10	<1	>5.7	33	5.1	7.0	<10	<1	>5.7
25	35	5.1	7.0	<10	<1	>5.7	35	5.0	7.0	<10	<1	>5.7

electric current (34–38, 41–43), to our knowledge there is no study of the mechanism of haloarchaeal inactivation by alternating electric current. The mechanism of haloarchaeal inactivation by alternating electric current requires elucidation. This will necessitate additional studies for determination of disinfection byproducts arising after alternating electric current treatment. Also, the morphological and physiological effects of the treatment on the cells should be examined in order to clarify the inactivation mechanism of this process. With improved understanding of the uses of alternating electric treatment for haloarchaeal inactivation, this cheap and practical application is expected to prove itself useful as science and as a boon to the leather industry.The knowledge gap will be reduced further as the mechanisms of haloarchaeal inactivation by alternating electric current are analysed in forthcoming experiments.

5. Conclusions

The experimental results of this study proved that using 2 A alternating current may effectively kill extremely halophilic archaea, proteolytic extremely halophilic archaea and lipolytic extremely halophilic archaea in a time period between 1 min and 5 min. The extremely halophilic archaeal population in salt samples was killed in 1 min, and the extremely halophilic archaeal isolates were killed in 5 min. This alternating current treatment was found to be fairly effective in killing extremely halophilic archaea in salt. In conclusion, this cheaper and simpler inactivation treatment system compared to antimicrobial agent applications may be used in hide brine solutions to destroy proteolytic and lipolytic extremely halophilic archaea.

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References

1 M. Mwinyihija, *Adv. Bus. Management Admin*., 2014, **2**, (1), 1

2 "World Statistical Compendium for Raw Hides and Skins, Leather and Leather Footwear, 1993–2012", Market and Policy Analyses of Raw Materials, Horticulture and Tropical Products Team, Trade and Markets Division, Food and Agriculture Organization of the United Nations, 2013, 193 pp

3 M. Mwinyihija and W. Quisenberry, *Global Adv. Res. J. Management Bus.*, 2013, **2**, (11), 518

4 M. Mwinyihija, *Int. J. Manag*., 2014, **3**, (3), 6

5 "World Statistical Compendium for Raw Hides and Skins, Leather and Leather Footwear 1992–2011", Food and Agriculture Organization of the United Nations (FAO), Commodities and Trade Division, Viale delle Terme di Caracalla, Rome, Italy, 2011

6 D. G. Bailey, *J. Am. Leather Chem. Assoc.*, 2003, **98**, (8), 308

7 E. Aslan and M. Birbir, *J. Am. Leather Chem. Assoc*., 2011, **106**, (12), 372

8 E. Aslan and M. Birbir, *J. Am. Leather Chem. Assoc*., 2012, **107**, (4), 106

9 W. D. Grant, M. Kamekura, T. J. Mcgenity and A. Ventosa, 'Phylum All. Euryarchaeota *phy. nov.,* Class III. Halobacteria, Order I. Halobacteriales', in "Bergey's Manual of Systematic Bacteriology: The Archaea and the Deeply Branching and Phototrophic Bacteria", 2nd Edn., eds. D. R. Boone, R. W. Castenholz and G. M. Garrity, Springer-Verlag, New York, USA, 2001, Vol. 1, pp. 294–333

10 A. Ventosa, 'Unusual Micro-organisms from Unusual Habitats: Hypersaline Environments', in "Symposium 66: Prokaryotic Diversity: Mechanisms and Significance", eds. N. A. Logan, H. M. Lappin-Scott and P. C. F. Oyston, Cambridge University Press, Cambridge, UK, 2006, p. 224

11 M. Birbir, B. Calli, B. Mertoglu, R. E. Bardavid, A. Oren, M. N. Ogmen and A. Ogan, *World J. Microbiol. Biotech*., 2007, **23**, (3), 309

12 W. E. Kallenberger, *J. Am. Leather Chem. Assoc*., 1984, **79**, (3), 104

13 D. J. Lloyd, R. H. Marriott and M. E. Robertson, *J. Internat. Soc. Leather Trades' Chem.*, 1929, **13**, (11), 538

14 F. J. Post, *Microbial Ecol.,* 1977, **3**, (2), 143

15 A. Oren, 'The Microbial Ecology of the Dead Sea', in "Advances in Microbial Ecology", ed. K. C. Marshall, Plenum Press, New York, USA, 1988, Vol. 10, pp. 193–229

16 W. D. Grant and B. J. Tindall, 'The Alkaline Saline Environment' in "Microbes in Extreme Environments", eds. R. A. Herbert and G. A. Codd, Academic Press, London, UK, 1986, pp. 25–54

17 M. B. Mutlu, M. Martínez-García, F. Santos, A. Peña, K. Guven and J. Antón, *FEMS Microbiol. Ecol*., 2008, **65**, (3), 474

18 D. Berber and M. Birbir, *J. Am. Leather Chem. Assoc.*, 2010, **105**, (10), 320

19 D. G. Bailey and M. Birbir, *J. Am. Leather Chem. Assoc.*, 1993, **88**, (8), 291

20 M. Birbir, *J. Turkish Microbiol. Society*, 1997, **27**, (1–4), 68

21 D. G. Bailey and M. Birbir, *J. Am. Leather Chem. Assoc.*, 1996, **91**, (2), 47

22 R. H. Vreeland, D. G. Bailey and R. W. Claunch, US Agriculture, 'Method of Using Bile Salts to Inhibit Red Heat in Stored Brine-Cured Hides and Skins', *US Patent* 5,945,027; 1999

23 B. O. Bitlisli, H. A. Karavana, B. Basaran, O. Sarı, I. Yasa and M. Birbir, *J. Am. Leather Chem. Assoc.*, 2004, **99**, (12), 494

24 C. P. Davis, N. Wagle, M. D. Anderson and M. M. Warren, *Antimicrob. Agents Chemother.*, 1991, **35**, (10), 2131

25 T. Matsunaga, S. Nakasono, T. Takamuku, J. G. Burgess, N. Nakamura and K. Sode, *Appl. Environ. Microbiol.*, 1992, **58**, (2), 686

26 A. J. H. Sale and W. A. Hamilton, *Biochim. Biophys. Acta.*, 1967, **148**, (3), 781

27 X.-G. Li, H.-B. Cao, J.-C. Wu and K.-T. Yu, *Biotechnol. Lett.*, 2001, **23**, (9), 705

28 J.-C. Park, M. S. Lee, D. H. Lee, B. J. Park, D.-W. Han, M. Uzawa and K. Takatori, *Appl. Environ. Microbiol.*, 2003, **69**, (4), 2405

29 Y. Birbir, G. Uğur and M. Birbir, *J. Electrostat.*, 2008, **66**, (7–8), 355

30 Y. Birbir, D. Degirmenci and M. Birbir, *J. Electrostat.*, 2008, **66**, (7–8), 388

31 M. Birbir, B. Ozdoğru, Y. Birbir and A. Ogan, *J. Soc. Leather Technol. Chem.,* 2008, **92,** (2), 53

32 A. K. Mahapatra, D. L. Harris, C. N. Nguyen and G. Kannan, *J. Electrostat.*, 2011, **69**, (1), 30

33 M. F. Slavik, C. Griffis, Y. Li and P. Engler, *J. Food Prot.*, 1991, **54**, (7), 508

34 K. Shimada and K. Shimahara, *Agric. Biol. Chem.*, 1982, **46**, (5), 1329

35 J.-C. Park, M. S. Lee, D.-W. Han, D. H. Lee, B. J. Park, I.-S. Lee, M. Uzawa, M. Aihara and K. Takatori, *Appl. Environ. Microbiol.*, 2004, **70**, (3), 1833

36 A. Pareilleux and N. Sicard, *Appl. Microbiol.*, 1970, **19**, (3), 421

37 R. L. Tracy Jr., *J. Bacteriol.*, 1932, **24**, (6), 423

38 K. Thamaphat, C. Oopathump and B. Panacharoensawad, *J. Appl. Sci.*, 2010, **10**, (24), 3222

39 K. P. Drees, M. Abbaszadegan and R. M. Maier, *Water Res.*, 2003, **37**, (10), 2291

40 J. C. Weaver and Yu. A. Chizmadzhev, *Bioelectrochem. Bioenerg.*, 1996, **41**, (2), 135

41 K. Shimada and K. Shimahara, *Agric. Biol. Chem.*, 1985, **49**, (12), 3605

42 K. Shimada and K. Shimahara, *Agric. Biol. Chem.*, 1985, **49**, (2), 405

43 K. Shimada and K. Shimahara, *Agric. Biol Chem.*, 1991, **55**, (5), 1247

44 C. Sánchez-Porro, S. Martín, E. Mellado and A. Ventosa, *J. Appl. Microbiol.*, 2003, **94**, (2), 295

45 R. Rohban, M. A. Amoozegar and A. Ventosa, *J. Ind. Microbiol. Biotechnol.*, 2009, **36**, (3), 333

46 Y. Birbir and M. Birbir, *J. Electrostat.*, 2006, **64**, (12), 791

47 M. Birbir, H. Hacıoğlu, Y. Birbir and G. Altuğ, *J. Electrostat.*, 2009, **67**, (4), 640

48 Y. Birbir, S. Molla and M. Birbir, *J. Soc. Leather Technol. Chem.*, 2013, **97**, (1), 5

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