Application of Alternating Electric Current in Leather Industry to Kill Extremely Halophilic Archaea Found in Brine Solutions

Yasar Birbir
Department of Electric and Electronic Engineering, Faculty of Technology, Marmara University, Goztepe, Istanbul, Turkey
Email: ybirbir@marmara1.edu.tr

Sema Anik [1], Meral Birbir [2], and Pinar Caglayan [2]
[1] Institute of Pure and Applied Sciences, Marmara University, Goztepe, Istanbul, Turkey
[2] Division Plant Diseases and Microbiology, Department of Biology, Faculty of Arts and Sciences, Marmara University, Goztepe, Istanbu, Turkey
Email: {semaanik, m.birbir64, marpdora_py}@hotmail2.com

***Abstract*—The hides are cured with salt obtained from Tuz Lake in Turkish Leather Industry. Salt obtained from natural salt sources contains extremely halophilic archaea and these microorganisms reduce the quality of leather. Most of antibacterial agents used in leather industry cannot effectively inactivate extremely halophilic archaea because of the high salt concentration in brine solutions. The goal of this study is to determine an alternative treatment system in order to inactivate extremely halophilic archaea in the brine solution. Brine samples were collected from Tuz Lake and the total cell number of extremely halophilic archaea was determined. 2A alternating electric current was implemented to the brine samples for 25 minutes. Brine samples contained usually 10^3-10^4 colony forming unit of extremely halophilic archaea per mL. One minute of 2A alternating electric current treatment was enough to eradicate extremely halophilic archaea in brine solutions and this can reduce economic losses in leather industry.**

***Index Terms*—alternating electric current, leather industry, extremely halophilic archaea**

I. Introduction

Salt lakes, salterns, salt mines and saline soil harbor different species of extremely halophilic archaea. These microorganisms play a pivotal role in these environments with thousand of different species. Solar salt production is mostly accomplished by the placing brine in shallow ponds where the sun evaporates most of water and concentrated salt is produced. Then, this salt is used for hide preservation in leather industry. In Turkey, salt produced from Tuz Lake, located in Central Anatolia, is used to cure hides. The brine samples collected from Tuz Lake and Kaldirim Saltern were found as 10^3-10^5 CFU/mL and 10^5 CFU/mL, respectively [1].

When the salt curing method is applied to hides, extremely halophilic archaea in salt may grow in high numbers in the salted hides. In the previous study in which 36 salt-pack cured hide samples examined, extremely halophilic archaea were found between 10^3-10^8 CFU/g [2]. Due to an inadequate salt curing preservation method, all hides contained extremely halophilic archaea in high numbers [2], [3]. Berber and Birbir (2010) notified that while 94% of the samples contained proteolytic extremely halophilic archaea (10^2-10^6 CFU/g), 81% of the samples contained lipolytic extremely halophilic archaea (10^2-10^6 CFU/g) [2]. The researchers reported that the extremely halophilic archaeal counts were found as 10^5-10^8 CFU/g in the brine cured hides [4], [5]. It can be seen that those results are consistent with each other.

It was explained that the grain surface and collagen fibers of hide are digested by proteolytic archaea originated from unprocessed solar salt by the researchers [6]. Moreover, it was mentioned that the extremely halophilic archaea, secreting protease and lipase, produced sponge-like vesicles within hide and light stains on the suede surface of finished double-face leathers [7], [8]. Due to the fact that the large archaeal community in salt, alternative methods should be applied for killing extremely halophilic archaea in salt directed for use in the leather industry. One of these methods is electric current treatment. Electric current was implemented by investigators for killing various microorganisms found in fresh orange juice [9], water [10], effluent seawater [11], soak liquors [12], brine solution, seawater and river water [13], [14]. In those studies, the investigators applied direct electric current to kill microorganisms.

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While it was showed that direct electric current can eliminate many species of microorganisms in different environments [9]-[15], the killing impact of alternating electric current on extremely halophilic archaea has not yet been reported. Therefore, the goal of this investigation was to examine the inactivation efficiency of alternating electric current on extremely halophilic archaea in brine used in hide preservation.

II. Materials and Methods

A. *Determination of Total Extremely Halophilic Archaeal Cell Numbers in the Brine Samples*

Five brine samples were obtained from Tuz Lake, and the viable extremely halophilic archaeal cell numbers in these samples were determined by plate count method. 20 mL of brine samples were placed into an electrolysis cell containing 180 mL of 25% NaCl. 100 µL of the test medium was removed from the electrolysis cell before the experiments, and was diluted to 10^{-1}, 10^{-2} and 10^{-3} with sterilized 25% NaCl solution. To determine the total extremely archaeal cell numbers in the brine samples, the direct and the diluted solutions were spread over complex media and incubated at 39°C for 25 days. After the incubation period, the colonies on the agar surface were counted [16].

B. *Inactivation of Extremely Halophilic Archaea in Brine Samples Using Alternating Electric Current*

A glass beaker having 2 internally attached platinum wire electrodes was used as an electrolysis cell. Liquid brine solution was placed into the electrolysis cell. Both electrodes were 1 mm in diameter and 80 mm in length located 40 mm apart from each other.

They were connected to a regulated alternating current (AC) source (Ruhstrat VDE Normadain Germany (Input=220V, f=50Hz, VA=2250VA), which had an automatic variable output voltage range of 0-220 V and user-selectable current range of 0-9 A (Fig. 1). The current levels were set at 2 A and applied into the brine solution for 1, 5, 10, 15, 20 and 25 min [11], [17].

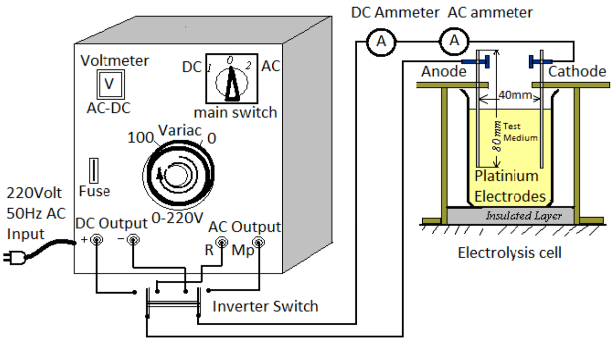

Figure 1. Schematic diagram of electrolysis cell sytem for brine samples, R: phase, Mp: ground.

III. Results and Discussion

The pH values of the brine samples examined in the present study were 7.0, which is optimum for the growth of extremely halophilic archaea (Tables I-II). The pH values of test media did not change during the experiment and was measured as 7.0.Voltage levels of the brine samples tested varied from sample to sample and exposure time with electric current.

At the end of experiment, voltage leves reduced. The temperature of all test media was 25°C before experiment. The temperature of all media was measures as 35°C when the experiment finished. Temperature of the test medium did not change when the extremely halophilic archaea was killed (Tables I-II).

TABLE I. pH and Temperature Values, Voltage Levels of the Brine Sample-1, Brine Sample-2, and Brine Sample-3 during Electric Treatment

	BS1[d]			BS2			BS3		
	pH	V	°C	pH	V	°C	pH	V	°C
BE[a]	7.0	-	25	7.0	-	25	7.0	-	25
ET[b] 1	7.0	5.0	25	7.0	5.6	25	7.0	5.4	25
5	7.0	4.8	27	7.0	5.4	27	7.0	5.3	27
10	7.0	4.7	29	7.0	5.3	29	7.0	5.2	29
15	7.0	4.5	30	7.0	5.2	30	7.0	5.1	30
20	7.0	4.4	32	7.0	5.1	32	7.0	5.0	32
25	7.0	4.4	35	7.0	5.0	35	7.0	4.9	35

[a] Before Experiment, [b] Exposure Time, [c] First Applied Voltage, [d] Brine Sample

TABLE II. pH and Temperature Values, Voltage Levels of the Brine Sample-4 and Brine Sample-5 during Electric Treatment

	BS4			BS5		
	pH	V	°C	pH	V	°C
BE	7.0	-	25	7.0	-	25
ET 1	7.0	7.5	25	7.0	7.6	25
5	7.0	7.4	27	7.0	7.5	27
10	7.0	7.4	29	7.0	7.5	29
15	7.0	7.3	30	7.0	7.3	31
20	7.0	7.3	32	7.0	7.2	33
25	7.0	7.2	35	7.0	7.1	35

The data of the present study showed similarity with our previous studies. Researchers found the pH values of Tuz Lake as 7.02 [18]. In this study, extremely halophilic archaea were detected in all brine samples. Brine samples contained 10^3 and 10^4 CFU of extremely halophilic archaea per mL (Tables III-VII). The results obtained from this study are similar to our previous studies [2], [13]. The researchers examined 80 salt samples in terms of extremely halophilic archaea and they explained that all salt samples contained extremely halophilic archaea between 10^2 and 10^5 CFU/g [2], [13]. According to the experimental results of electric current treatment, all extremely halophilic archaeal populations in brine solutions were killed in one min by 2A alternating electric current. Log_{10} reduction factors of extremely halophilic archaea in BS1, BS2, BS3, BS4 and BS5 were 4.45, 4.64, 3.50, 3.57 and 3.58, respectively (Table III-VII).

TABLE III. VALUES OF TOTAL EXTREMELY HALOPHILIC ARCHAEAL COUNTS (CFU/ML), LOG_{10} AND REDUCTION FACTORS OF EXTREMELY HALOPHILIC ARCHAEA IN THE BRINE SAMPLE-1 DURING THE ELECTRIC TREATMENT

ET[e] (min)	Brine Sample-1		
	Total numbers of extremely halophilic archaea	Log_{10} values of total extremely halophilic archaea	RF values of total extremely halophilic archaea
BE	$2.8x10^4$	4.45	-
1	-	-	4.45
5	-	-	4.45
10	-	-	4.45
15	-	-	4.45
20	-	-	4.45
25	-	-	4.45
[e]ET: Exposure Time			

TABLE IV. VALUES OF TOTAL EXTREMELY HALOPHILIC ARCHAEAL COUNTS (CFU/ML), LOG_{10} AND REDUCTION FACTORS OF EXTREMELY HALOPHILIC ARCHAEA IN THE BRINE SAMPLE-2 DURING THE ELECTRIC TREATMENT

ET[e] (min)	Brine Sample-2		
	Total numbers of extremely halophilic archaea	Log_{10} values of total extremely halophilic archaea	RF values of total extremely halophilic archaea
BE	$4.4x10^4$	4.64	-
1	-	-	4.64
5	-	-	4.64
10	-	-	4.64
15	-	-	4.64
20	-	-	4.64
25	-	-	4.64

TABLE V. VALUES OF TOTAL EXTREMELY HALOPHILIC ARCHAEAL COUNTS (CFU/ML), LOG_{10} AND REDUCTION FACTORS OF EXTREMELY HALOPHILIC ARCHAEA IN THE BRINE SAMPLE-3 DURING THE ELECTRIC TREATMENT

ET (min)	Brine Sample-3		
	Total numbers of extremely halophilic archaea	Log_{10} values of total extremely halophilic archaea	RF values of total extremely halophilic archaea
BE	$3.18x10^3$	3.50	-
1	-	-	3.50
5	-	-	3.50
10	-	-	3.50
15	-	-	3.50
20	-	-	3.50
25	-	-	3.50

In the previous studies, researchers examined the inactivation of Gram negative bacteria using alternating electric current [15], [19]. *Vibrio parahaemolyticus* in effluent seawater was inactivated by 3A alternating electric current (AC) treatment in 30ms [19]. In our earlier study, 1.5A 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 the hides. Fifteen minutes exposure to 1.5A alternating current inactivated all of the test bacteria in the brine solution containing 25% NaCl.

TABLE VI. VALUES OF TOTAL EXTREMELY HALOPHILIC ARCHAEAL COUNTS (CFU/ML), LOG_{10} AND REDUCTION FACTORS OF EXTREMELY HALOPHILIC ARCHAEA IN THE BRINE SAMPLE-4 DURING THE ELECTRIC TREATMENT

ET (min)	Brine Sample-4		
	Total numbers of extremely halophilic archaea	Log_{10} values of total extremely halophilic archaea	RF values of total extremely halophilic archaea
BE	$3.7x10^3$	3.57	-
1	-	-	3.57
5	-	-	3.57
10	-	-	3.57
15	-	-	3.57
20	-	-	3.57
25	-	-	3.57

TABLE VII. VALUES OF TOTAL EXTREMELY HALOPHILIC ARCHAEAL COUNTS (CFU/ML), LOG_{10} AND REDUCTION FACTORS OF EXTREMELY HALOPHILIC ARCHAEA IN THE BRINE SAMPLE-5 DURING THE ELECTRIC TREATMENT

ET[e] (min)	Brine Sample-5		
	Total numbers of extremely halophilic archaea	Log_{10} values of total extremely halophilic archaea	RF values of total extremely halophilic archaea
BE	$3.8x10^3$	3.58	-
1	-	-	3.58
5	-	-	3.58
10	-	-	3.58
15	-	-	3.58
20	-	-	3.58
25	-	-	3.58

Moreover, exposure to 1.5A alternating electric current for 15 minutes followed by 1.5A direct current for 1 minute inactivated the mixed population of these bacteria in the brine solution. The maximum temperature rise was 6 ℃ [15].

In conclusion, 2A alternating electric current treatment was found to be fairly effective in killing extremely halophilic archaea in brine samples. The use of 2A alternating electric current may prevent extremely halophilic archaeal damage during storage of salted hides.

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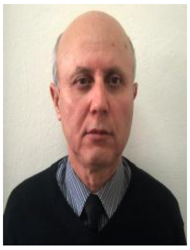

Yasar Birbir, received B.S degree from Gazi University, M.S and PhD. from Marmara University. He attended World Bank Industrial Training Project at Indiana and Purdue Universities from 1989 to 1990. He had worked as a visiting research scientist for fifteen months at Drexel University Electrical and Computer Engineering Department from 1992 to 1993. Currently he has been working as an Associate Professor at Technology Faculty in Department of Electrical Engineering. He teaches undergraduate and graduate courses in Power Electronics Courses and Electrical Machinery Drives. His current interests beside power electronic converters, drivers, electromagnetic filtering process in the industry. The application of electric currents and electric field effects for sterilization of different microorganisms are his research subjects. He has published 15 research articles and graduated 12 masters and one doctorate students. He presented 30 oral or poster presentations in national and international congress. He has completed 5 scientific projects.

Meral Birbir, received a bachelor's degree in biology education in 1985, M.S. and Ph.D. degrees in 1997 and 1997, respectively from Department of Biology, Marmara University. She has been working at Biology Department of Marmara University since 1985. She was a visiting research scientist in Department of Pathology and Microbiology, Veterinary Medical School, Purdue University in 1990. She was a research scientist in Hides and Leather Department of USDA from 1992 to 1993. She is Erasmus Coordinator of Biology Department, Marmara University since 2006. She teaches undergraduate and graduate courses in biology, microbiology, clinic microbiology and industrial microbiology. Her research interests are in halophilic bacteria, hide microbiology, food microbiology, antimicrobial agents, electric current application and microbiology of hypersaline environment. Professor Birbir especially focused on halophilic and non-halophilic bacteria that live on salted hides and their control with antimicrobials or electric current. She has published 53 research articles and graduated 28 masters and one doctorate students. She presented 46 oral or poster presentations in national and international congress. She has completed 23 scientific projects.

Sema Anik, graduated from both Department of Biology and Chemistry, Faculty of Arts and Sciences, Marmara University, Türkiye in 2010. She received her M.Sc. degree in Biology in 2014 from Institute for Graduate Studies in Pure and Applied Science, Marmara University. She worked as a researcher in a clinic microbiology laboratory. She has been working as a biology teacher in private high school. Her research experience is controlling growth of extremely halophilic archaea using electric current applications.

Pinar Caglayan, graduated from Department of Biology, Ataturk Faculty of Education, Marmara University, Turkiye in 2007. She received her M.Sc. degree in Biology in 2010 from Institute of Pure and Applied Sciences, Marmara University. She was an Erasmus student in Department of Microbiology and Parasitology, Faculty of Pharmacy, Sevilla University, Spain from 2008 to 2009 and 2013. Pınar Caglayan is Graduate student (PhD) at Marmara University, Department of Biology. She has been working as a research and teaching assistant of the Division of Plant Diseases and Microbiology, Department of Biology, Faculty of Science and Letters, Marmara University since 2011. Her research interests are moderately halophilic bacteria, extremely halophilic Archaea, antimicrobial agents, electric currents and hide microbiology. She has published 4 research articles. She presented 8 poster presentations in international congress. She is still working in a scientific project as a researcher at Marmara University.