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EFFECTS OF LOW VOLTAGE ELECTROLYSIS AND FREEZING ON COLIFORM CONTENT OF CONTAMINATED WATER

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Abstract: A sewage sample was mixed with drinking water and subjected to low voltage (15V) electrolysis in the presence of 1% NaCl. The prepared sample was also kept in freezer with and without the presence of sodium chloride for 4-hours. Among these treatments the electrolysis proved to kill the coliforms, while the freezing reduced the bacterial content. Antibiotics sensitivity patterns revealed that certain of the coliform strains survived the freezing and thawing shocks. Nature of such surviving bacteria and need to study chemical parameters of electrolyzed water are discussed.

Keywords: Antibiotic susceptibility of coliforms, coliforms and electric current, electrolysis and water treatment, freezing and water treatment, NaCl and coliforms.

INTRODUCTION

Cases of water born infectious diseases depend on the prevalence of microbes and contents and physical characteristics of water that may enhance the growth of the pathogens [Reacher *et al*. 1999, Lobitz *et al*. 2000]. A large proportion of the world's population is currently experiencing water stress [Vörösmarty *et al*. 2000]. Increase in consumption due to modern lifestyle, more chances of contamination of recreational water supply with sewage and waste water and its recognition as scarce resource in future have necessitated high demands of potable water provisions [Brown 2001].

Most of the urban environment of Pakistan is deprived of proper wastewater treatment facilities and rather it may contaminate storage and/or supplies of drinking water. In the rural areas shallow ponds, streams, boreholes, wells and other water supplies may harbor pathogenic microorganisms [Yulug and Tug 1988, Reacher *et al*. 1999, Licence *et al*. 2001]. Even following municipal treatments, contamination and growth supporting conditions may prevail in post-treatment storage and supply. For example, Camper *et al*. [1991] indicated that growth of environmental coliforms is possible under the conditions found in operating municipal drinking water system and that these bacteria could be used in tests to determine assimilable organic carbon in potable water. Monitoring of coliforms in drinking water has been used extensively as an indicator of water quality [Rompre *et al*. 2002]. Apart from known municipal drinking water treatment facilities and a variety of costly filtration and/or UV mediated, boiled and mineralized potable water provisions there is acute need to design low cost strategies for microbiological purification of water. In this regard many attempts have been reported including filtration [Pelczar *et al*. 1986, Black 1996] and freezing [Dilek *et al*. 1994].

This work was intended to see the effects of low voltage electrolysis and freezing in the presence of NaCl upon the fate of coliform bacterial contamination of a water sample. Although lethal effects of electric current in microorganisms have long been reported. For instance, Hulsheger and Niemann [1980] have reported lethal effect of high voltage capacitor discharger in suspension of *E. coli*. Similarly, Hulsheger *et al*. [1981] discussed the role of length of electric pulses, field strength, pH and temperature on killing of the bacteria, while Kakez *et al*. [1996] proposed that lethal breakdown of microorganisms suspended in a continuous medium depends primarily on strength of electric field and the joules energy deposited in the membrane of microbes. The present study was aimed at designing low voltage electrolysis for killing coliform bacteria of water that may reduce cost and make the process safer as compared to the use of high voltage current. Besides, a highly appealing facet of electrolyzed water is that disinfection against viral pathogens such as B-virus and HIV is achieved [Morita *et al*. 2000] and obviously viruses escape from different microbial filtration strategies. Another aspect of this work was to compare the microbial pictures of the water with and without 1% NaCl following exposure to freezing temperature for a few hours. The data obtained in this study are suggestive to use electrolysis and freezing for microbiological water treatment processes.

MATERIALS AND METHODS

Water sample was prepared by mixing 0.5% sewage effluent in autoclaved tap water. One percent NaCl was added to a part of water sample and divided into two portions. For electrolysis a sterile glass container of 5.9 cm internal diameter and 12 cm height, fitted with a hard plastic cover was used. DC current of 15 volts was passed for 15 minutes through 150 ml of the water. Each of the two copper electrodes comprised of 10 cm wire coiled into 2.5 concentric loops with a diameter of 2 cm for the outermost one. The pH of sample before and after the electrolysis was noted. The water sample containing 1% NaCl was also subjected to freezing temperature in an electric freezer for four hours. Control and the treated water samples were processed for bacteriological analyses as mentioned below.

WATER ANALYSIS

Water sample was analyzed for multiple tube most probable number (MPN) fermentation technique [Benson 1994, Collins *et al*. 1995]. For the presumptive test single strength (SSB) and double strength (DSB), brilliant green bile broths, were prepared according to Merck [1996/97]. Three sets, each of three test tubes, fitted with Durham tubes were employed for a given sample. One of the sets was dispensed with 10 ml of DSB-test tube. The other two sets were dispensed, similarly with the same amount of SSB. Following autoclaving at 121°C for 15 min., DSB test tubes were inoculated with 10 ml, one set of SSB with 1 ml and the other with 0.1 ml of a water sample. Gas production was noted after 24 and 48 hours of incubation at 37°C and MPN of coliforms were determined from MPN table [Collins *et al*. 1995]. For confirmed test, MacConkey agar [Merck 1996/97] was prepared, autoclaved and poured in presterile Petri plates. Culture from brilliant green bile broth was spread and the agar plates were incubated for 24 hours at 37°C. The bacterial colonies from the plates were streaked on MacConkey agar to get separated colonies to note the colonial characteristics. These bacterial colonies were also subcultured on nutrient agar slants. Following incubation at 37°C for 24 hours, growth on nutrient agar slants was processed for Gram's staining, size determination of the bacteria, completed test, antibiotics sensitivities and physiological characterization based upon indole, methyl red, Voges-Proskauer and citrate utilization tests ((IMVIC) according to Benson [1994].

Table 1: Effects of 1% NaCl (S), electrolysis (ES)[a], freezing (F) and freezing in presence of the salt (FS) on coliforms of water sample.

Experiment	Strain No.	Completed test	Size of bacteria (μm)	Colonial characteristics[b]
Control	C-1	+	1.37-4.21 (0.94)[c]	L, P, Mu, Tl, En, Rd, PS_o
	S-1	-	1.26-3.68 (0.94)	L, R, Mu, En, Rd, PS_o
S	S-2	+	1.26-4.21 (0.94)	L, LP, R, Mu, En, Rd, PS_o
	S-3	+	1.47-4.42 (0.84)	L, R, Mu, En, Rd, Tz.
	F-1	-	1.26-3.68 (0.84)	M, LP, Mu, Tl_p, En, Rd, PS_o
F	F-2	+	1.16-2.63 (0.74)	L, LP, Mu, En, Con.
	F-3	+	1.16-3.47 (0.84)	L, LP, Mu, En, Con.
FS	FS-1	+	1.26-4.21 (0.84)	L, P, Mu, Tl_p, En, Con.
	FS-2	-	1.05-2.42 (0.84)	L, LP, Mu, Tl_p, En, Con.

a= The electrolysis proved bactericidal and no coliforms were detected; b= On MacConkey agar; c= Values indicate range of bacterial length and those within parenthesis show cells' width.
L= large; P= pink; LP= light pink; R= red; PS= pigmented spots; Mu= mucoid; En= entire margin; Tz= turbid zone; Con= convex elevation; Rd= raised elevation; Tl= translucent; the subscript "o" indicates that the characteristic was confined to the center of a colony while subscript "p" indicates its presence in the peripheral zone.

RESULTS

The prepared sample was found to harbor more than 1100 figures for MPN of coliforms. Presence of one percent NaCl did not affect the content of coliforms as compared to the control sample. However, following electrolysis MPN dropped to zero, while freezing in the presence and absence of 1% NaCl, dropped MPN from > 1100 to 1100 Electrolysis of the water was accompanied by increase in pH units from 8.5 to 11 and precipitation of some salts. Results of completed test and characterization of strains indicated that the control sample harbored only one strain (C-1) of coliform. Of the three strains isolated from the sample water containing 1% NaCl, two were confirmed coliforms, while in case of 1% NaCl freezed water one out of two and in case of freezed but without the salt two out of three strains showed positive completed test. The bacteria other than the coliform were also found Gram-negative rods of different dimensions (Table 1).

Table 2: Antibiotics sensitivity/resistance patterns of various strains of coliforms isolated from control, containing 1% NaCl (S), freezed (F) and the salt containing freezed (FS) water sample.

Isolation sample	Bacterial strains	Antibiotics					
		S_{10}	PRL_{100}	MTZ_5	C_{30}	TE_{30}	E_{15}
Control	C-1	16.0[a]	17.0	–[b]	21.5	9.5	11
	S-1	12.0	8.0	–	–	–	–
S	S-2	11.0	8.5	–	–	–	–
	S-3	12.5	8.5	–	–	–	–
	F-1	9.0	16.5	–	18.0	–	–
F	F-2	13.0	–	–	10.5	–	–
	F-3	22.0	13.0	–	22.0	10.0	11.0
FS	FS-1	10.5	19.0	–	–	–	9.5
	FS-2	23.0	18.0	–	27.0	13.0	18.5

a= Diameter (mm) of zone of growth inhibition, b= bacteria were found resistant to antibiotic, S= Streptomycin, PRL= Piperacillin, MTZ= Metronidazol, TE= Tetracycline, C= Chloramphenicol, E= Erythromycin. Subscripts indicate potency (μg) of antibiotics on each diskette.

Colonial features (Table 1) of the bacteria permitted to consider strains S-3 as *E. coli*, while C-1, FS-1, FS-2, F-1 to F-3 as *Enterobacter/ Klebsiella*. Concerning the biochemical tests all the strains were positive for Voges-Proskauer and citrate tests and negative for methyl red test. Regarding indole test only FS-1 and FS-3 appeared negative, while all the remaining strains showed positive results (Table 1). When these strains were tested for antibiotics sensitivity tests (Table 2) all were found resistant to antibiotic MTZ. Strains S-1 to S-3 and FS-1 were found resistant to antibiotic C, while S-1 to S-3, FS-1, F-1 and F-2 appeared resistant to antibiotic TE (Table 2). All strains except F-2 showed resistance to antibiotic PRL, whereas for the drug E, strains S-1 to S-3, F-1 and F-2 appeared resistant (Table 2). The coliform of the water sample remained resistant to metronidazole in control as well as following the freezing treatment. However, all the strains from the water containing 1% NaCl appeared resistant to the antibiotics chloramphenicol, tetracycline and erythromycin. Of the strains isolated from the water following freezing in the absence and presence of NaCl, some showed resistance to different antibiotics except the streptomycin. In a recent investigation Kolar *et al.*

[2001] while studying antibiotic resistance in Gram-negative rods to various antibiotics at University Hospital in Olomouc, Czech Republic, have demonstrated that development of bacterial resistance was linked with antibiotic use. They found that selective pressure was specific for the type of antibiotic and bacterial species. Resistant coliforms to various antibiotics in present study might have correlations to the frequencies of uses of the corresponding antibiotics.

DISCUSSION

Electrolysis of the water sample apparently gave rise to very appealing results. Passage of 15 volts of current through the water in the presence of 1% NaCl killed all the coliforms within 15 minutes accompanied by an increase of pH from 8.50 to 11.00. Hulsheger and Niemann [1980] have described that high voltage caused a reduction of more than 99.9% of *E. coli* cells. These workers suggested the bactericidal action due to direct effect of high electric fields and considered production of chlorine during the process as an additional toxic agent. In the same study relative survival rate of bacteria was found to depend on the concentration of cells during pulse treatment. Detailed studies are needed to focus fate of varying contents of coliforms following electrolysis. Such information will be of value in designing electrolysis-based water treatment facility.

Nature of electrolyte(s) involved in the process of electrolysis has bearing on its lethal effects on bacteria. In this regard 100% mortality of coliforms in the present study was achieved in the presence of 1% NaCl. Hulsheger *et al*. [1981] have reported that electrolytes with bivalent cation reduced lethal action on bacteria. These workers also indicated that the process is governed by field strength, pH and the treatment time. Obviously, there is need to extend studies involving also alterations in voltage, time, pH and other features of the water to be treated.

Regarding the effect of keeping water samples in freezing temperature for 4 hours with subsequent thawing, interesting results were obtained. It appeared that freezing with and without 1% NaCl reduced the MPN figure to 1100, but due to very high initial level of coliform (>1100) the % reductions could not be calculated. Freeze/thawed sludge conditioning has been shown to reduce pathogens [Dilek *et al*. 1994]. In Pakistan, many high altitude areas are characterized by freezing temperatures, but with scarce fuel supply. So for such regions this simple management may prove effective in terms of improving quality of drinking water. The coliform of the water sample remained resistant to metronidazole in control as well as following the freezing treatments. However, in the presence of 1% salt all the strains were found resistant to the antibiotics chloramphenicol, tetracycline and erythromycin. Of the strains isolated from the water sample following freezing in the absence and presence of NaCl some showed resistance to different antibiotics except the streptomycin.

Some precipitates following the electrolysis in the present experiment might had appeared due to nature of the electrodes as well as water and electrodialysis of the microbes. Kekez *et al*. [1996] have demonstrated that lethal breakdown of microorganisms to occur, the applied electric field must exceed the critical field of membrane to create holes and the Joule energy deposited in the membrane must exceed the minimum value beyond which the cell cannot recover. Future studies should focus to improve the quality of electrodes and to assess the chemical nature of the precipitates as well as electrolyzed water. Especially the treated water should be assessed for endotoxin contents that might be released by the dead microbes in addition to other physicochemical characteristics.

As pressures on the world's freshwater resources has resulted to devise methods for recycling of water, many approaches varying between high technology, high cost, low risk and low technology/low cost are being considered to be linked together into international water recycling guidelines [Anderson *et al*. 2001]. The low voltage passage of electric current that proved bactericidal for the water coliform content in the present study may add to valuable technology in an integrated approach to recycle water.

References

Anderson, J., Adin, A., Crook, J., Davis, C., Hultquist, R., Jimenez-Cisneros, B., Kennedy, W., Sheikh, B. and van der Merwe, B. (**2001**) "Climbing the ladder: a step by step approach to international guidelines for water recycling", *Water Sci. Technol*., 43, 1-8.

Benson, H.J. (**1994**) "Microbiological Applications, Laboratory Manual in General Microbiology", Wm. C Brown Publishers, Dubuque, USA, pp.137-209.

Black, J.G. (**1996**) "Microbiology Principles and Applications", Prentice Hall, Upper Saddle River, New Jersey, pp. 350-351.

Brown, L.R. (**2001**) "How water scarcity will shape the new century", *Water Sci. Technol*., 43, 17-22.

Camper, A.K., McFeters, G.A., Characklis, W.G. and Jones, W.L. (**1991**) "Growth kinetics of coliform bacteria under conditions relevant to drinking water distribution systems", *Appl. Environ. Microbiol*., 57, 2233-2239.

Collins, C.H., Lyne, P.M. and Grange, J.M. (**1995**) "Microbiological Methods", Butterworth Heinemann Ltd., London, pp. 62-162.

Dilek, S.F., Aarne, V.P. and James, M.C. (**1994**) "Pathogen reduction capabilities of freeze/thaw sludge conditioning", *Water Res*., 28, 2393-2398.

Hulsheger, H. and Niemann, E.G. (**1980**) "Lethal effects of high-voltage pulses on *E. coli* K12", *Radiat. Environ. Biophys*., 18, 281-288.

Hulsheger, H., Potel, J. and Niemann, E.G. (**1981**) "Killing of bacteria with electric pulses of high field strength", *Radiat. Environ. Biophys.*, 20, 3-65.

Kekez, M.M., Savic, P. and Johnson, B.F. (**1996**) "Contribution to the biophysics of the lethal effects of electric field on microorganisms", *Biochim. Biophys. Acta*, 1278, 79-88.

Kolar, M., Urbanek, K. and Latal, T. (**2001**) "Antibiotic selective pressure and development of bacterial resistance [original article]", *Internat. J. Antimicrobial Agents*, 17, 357-363.

Licence, K., Oates, K.R., Synge, B.A. and Reid, T.M. (**2001**) "An outbreak of *E. coli* 0157 infection with evidence of spread from animals to man through contamination of a private water supply", *Epidemiol. Infect.*, 126, 135-138.

Lobitz, B., Beck, L., Huq, A., Wood, B., Fuchs, G., Faruque, A.S.G. and Colwell, R. (**2000**) "Climate and infectious disease: Use of remote sensing for detection of *Vibrio cholerae* by indirect measurement", *Appl. Biological Sci.*, 97, 1438-1443.

Merck (**1996/97**) "Microbiology Manual", pp. 70-163.

Morita, C., Sano, K., Morimatsu, S., Kiura, H., Goto, T., Kohno, T., Hong, W., Miyoshi, H., Iwasawa, A., Nakamura, Y., Tagawa, M., Yokosuka, O., Saisho, H., Maeda, T. and Katsuoka, Y. (**2000**) "Disinfection potential of electrolyzed solutions containing sodium chloride at low concentrations", *J. Virol. Methods*, 85, 163-174.

Pelczar, M.J., Chan, E.C.S. and Krieg, M.R. (**1986**) "Microbiology", McGraw Hill Book Company, New York, pp. 614-615.

Reacher, M., Ludlam, H., Irish, N., Buttery, R. and Murray, V. (**1999**) "Outbreak of gastroenteritis associated with contamination of a private borehole water supply", *Commun. Dis. Public Health*, 2, 27-31.

Rompre, A., Servais, P., Baudart, J., de-Roubin, M.R. and Laurent, P. (**2002**) "Detection and enumeration of coliforms in drinking water: current methods and emerging approaches", *J. Microbiol. Methods*, 49, 31-54.

Vörösmarty, C.J., Green, P., Salisbury, J. and Lammers, R.B. (**2000**) "Global water resources: Vulnerability from climate change and population growth", *Science*, 289, 284-288.

Yulug, N. and Tug, A. (**1988**) "Microbiological analysis of well water samples in the rural areas near Ankara", *Mikrobiyol. Bul.*, 22, 164-171.