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Comparative electrochemical inactivation of bacteria and bacteriophage

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Abstract

Electric fields and currents have been shown to be capable of disinfecting drinking water and reducing the numbers of bacteria and yeast in food. However, little research has been conducted regarding the effectiveness of electric fields and currents in the inactivation of viruses. The objective of this study was to compare the ability of bacteria and bacteriophage to survive exposure to direct electric current in an electrochemical cell, where they would be subject to irreversible membrane permeabilization processes, direct oxidation of cellular/viral constituents by electric current, and disinfection by electrochemically generated oxidants. Suspensions of the bacteria *Escherichia coli* and *Pseudomonas aeruginosa* and bacteriophage MS2 and PRD1 at both high (approximately 1×10^6 CFU or PFU/mL) and low (approximately 1×10^3 CFU or PFU/mL) population densities were exposed to currents ranging from 25 to 350 mA in 5 s pulses. Post-exposure plaque counts of the bacteriophage were proportionally higher than bacterial culturable counts at corresponding current exposures. *E. coli* and MS2 were then exposed to 5 mA for 20 min at both high and low population densities. The inactivation rate of *E. coli* was 2.1–4.3 times greater than that of MS2. Both bacteria and bacteriophage were more resistant to exposure to direct current at higher population densities. Also, amelioration of inactivation within the electrochemical cell by the reducing agent glutathione indicates the major mechanism of inactivation in the electrochemical cell is disinfection by electrochemically generated oxidants. The implications of these results are that technologies relying upon direct current to reduce the numbers of microbes in food and water may not be sufficient to reduce the numbers of potentially pathogenic viruses and ensure the safety of the treated food or water.

Keywords: Virus; Bacteriophage; Electrochemical; Inactivation; Direct; Current

1. Introduction

The inactivation of bacteria and yeast cells by electrochemical means has been well documented [1–7]. Several mechanisms have been proposed to account for the lethality of electrochemical exposure, including oxidative stress and cell death due to electrochemically generated oxidants, irreversible permeabilization of cell membranes by the applied electric field, and electrochemical oxidation of vital cellular constituents during exposure to electric current or induced electric fields.

Chemical oxidants are generated when electric current is applied to aqueous suspensions of microbes with immersed electrodes [1,8]. Electrolysis at the electrodes generates a variety of oxidants in the presence of oxygen, including hydrogen peroxide and ozone, as well as free chlorine and chlorine dioxide when chloride ions are present in the solution [1,8,9]. Such oxidants are responsible for most, but not all, of the lethality of the applied direct current [8]. Current research indicates that antimicrobial agents and electric current act synergistically to inactivate microbes [10,11].

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Electric fields are themselves harmful to cells. It has been shown that this is primarily due to the irreversible permeabilization of the cell membrane [12]. To summarize this work, experiments conducted on artificial bilayer lipid membranes indicate that a membrane exposed to an external electric field gathers charge much like a capacitor, and a transmembrane potential is induced. A short-lived steady-state current across the membrane is established when the membrane is fully charged, demonstrating an induced permeability of the membrane to hydrophilic molecules. This phenomenon is most fully explained by models involving the formation of transient pores in the membrane due to exposure to the external electric field. Two critical parameters influence the reversibility of this electropermeabilization: the magnitude of the induced transmembrane potential, and the duration of the exposure to the external electric field. For cells, transmembrane potentials above 1 V and longer pulse times lead to irreversible permeabilization and cell death. The transmembrane potential induced by an external electric field depends upon the radius of the cell membrane, with larger cells suffering a greater transmembrane potential from a given electric field. Hence, the magnitude of the field needed to inactivate yeast cells is generally lower than that needed to inactivate bacteria [2]. Death occurs due to either the formation of permanent pores and subsequent destabilization of the cell membrane, or loss of important cell components and destruction of chemical gradients via transport through transient pores [12]. If electrochemically generated oxidants are present, these pores may allow the oxidants free access to the interior of the cell, aiding the inactivation process.

Electric fields are also capable of destroying cells without destroying their membranes. Matsunaga et al. [4] describe a system in which cells were killed without rupturing, but rather with the electrochemical oxidation of intracellular coenzyme A. Thus, electric fields may directly oxidize cellular constituents, leading to cell death.

A great deal of research has focused upon the use of electric fields and currents to kill bacteria and yeast in industrial and medical applications, as illustrated by the following examples. Drinking water contaminated with *Escherichia coli* K12 (100 cells/cm^3) was disinfected at a rate of 600 cells/cm^3/h with the application of a 0.7 V electric potential using a carbon cloth electrode [4]. Drinking water contaminated with 335 cells/mL total coliforms and 1035 cells/mL fecal streptococci was sterilized with a 2.5 mA/cm^2 direct current density (125 mA current) applied with 5 cm × 5 cm titanium electrodes for 30 min [5]. Direct current (60 mA) was used to inhibit the growth of *E. coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* contaminants of a bioprocess reactor [6]. In a search for a non-thermal pasteurization process that would protect the vitamins, enzymes, texture, and taste of treated foods, the effects of pulsed electric fields on *E. coli* and *Saccharomyces cerevisiae* suspended in milk and fruit juice, respectively, were studied [3]. Wounds treated with 200–800 μA direct current healed 1.5–2.5 times faster than a control group, due in part to the bactericidal effect of the current application [13]. The population of a *Staphylococcus epidermitis* biofilm adhered to a catheter was reduced by the application of 100 μA direct current, suggesting that direct current can be used to inhibit biofilm formation on biomedical implants [8].

Few such studies have been conducted with viruses, however. One would expect viruses to be more resistant to electrochemical inactivation than bacteria. For example, some viruses (notably bacteriophage) are not enveloped with a membrane and would be immune to inactivation processes involving irreversible membrane permeabilization. Even enveloped viruses would be more resistant than their cellular counterparts due to their smaller size. Viruses also tend to be more resistant to chemical disinfectants, such as chlorine and ozone, than vegetative bacteria [14]. Recently, bacteriophage MS2 was suggested as a model for the study of the biological effects of electric fields, mainly due to its relatively simple biology compared to prokaryotic and eukaryotic cells [15]. When exposed to an induced 60 Hz electric field, a lag in MS2 yield was demonstrated, but the overall phage yield was totally unaffected [15]. Should viruses prove more resistant to electrochemical inactivation than bacteria as these results suggest, technologies using electric current to kill bacteria may not be sufficient to ensure treated food or drinking water is safe from viruses.

The objective of this study was to compare the ability of bacteria and bacteriophages to survive exposure to direct electric current in an electrochemical cell, where they would be subject to irreversible membrane permeabilization processes, direct oxidation of cellular/viral constituents by electric current, and disinfection by electrochemically generated oxidants. Suspensions of bacteriophage MS2 and PRD1, two commonly studied non-enveloped enterobacterial viruses, were exposed to direct current with a variety of magnitudes and durations of application. The survival of the bacteriophage to such treatments was compared to the survival of two species of bacteria: *E. coli* and *P. aeruginosa*, whose responses to direct electric current have been previously studied [4–6]. Furthermore, certain trials were supplemented with reduced glutathione (GSH), a molecule used by eukaryotic cells to protect themselves intracellularly and extracellularly from alkylating agents, free radicals, and oxidative stress [16]. The thiol group of GSH reduces oxidizing agents, preventing them from damaging the cell. GSH can protect cells by scavenging chemical oxidants, but cannot protect cells

from oxidation due directly to an applied electric current, or membrane permeabilization. A protective effect in trials containing GSH would establish the relative importance of electrochemically generated oxidants in the inactivation mechanism.

Bacteriophage MS2 and PRD1 were chosen as model viruses in this study due to the ease of their culture and assay methodology, and their gross morphological similarity to waterborne enteric viruses. Furthermore, the USEPA has cited such coliphages as acceptable indicators of enteric viruses in the proposed Ground Water Rule [17]. Thus, coliphages are assumed to survive disinfection processes to a similar degree as some, if not all, enteric viruses.

2. Materials and methods

2.1. Bacteriophage and bacterial strains

The effect of electric current on bacteriophage viability was tested on bacteriophage MS2, grown on its *E. coli* ATCC 15597 host, and bacteriophage PRD1, grown on *Salmonella choleraesuis* subsp. *choleraeusis* serotype Typhimurium ATCC 23564 host. The bacterial strains tested were *E. coli* ATCC 15224 and *P. aeruginosa* ATCC 9027.

2.2. Bacteria and bacteriophage culture and culturable counts

Bacterial strains were revived from glycerol freezer stocks by growth in nutrient broth (Difco Laboratories, Detroit, MI) on an orbital shaker at room temperature until turbid. From such revived cultures, *E. coli* ATCC 15224 was inoculated into tryptic soy broth (TSB; Sigma Chemical Co., St. Louis, MO) and incubated overnight as above before use. Likewise, *P. aeruginosa* ATCC 9027 was inoculated into R2A broth (Becton Dickinson & Co., Cockeysville, MD). Stationary phase bacterial cultures were used in these experiments because exponentially growing cells have been shown to be more sensitive to inactivation by electric fields [7]. Using the less sensitive stationary phase cells eliminated growth phase-dependent resistance to electric current exposure, and resulted in a more conservative comparison of electric current resistance between the bacteria and phage. Culturable counts for *E. coli* ATCC 15224 and *P. aeruginosa* ATCC 9027 were performed using the spread plate method on TSB or R2A agar plates, respectively. Plates were incubated at 37°C.

Log-phase host bacteria for bacteriophage propagation were grown in TSB as above. Before use, 100 μL of culture was inoculated into fresh TSB containing 0.0025% $CaCl_2$, which aids in the adsorption of phage to host cells. These cultures were incubated in a 37°C shaking water bath for 4 h until log phase was reached.

Fresh bacteriophages were cultured from freezer stocks using a modified version of the culture technique of Staczek et al. [15]. Log-phase host bacteria and phage were mixed with a multiplicity of infection (MOI) of approximately 1 in 5 mL TSB. The culture was kept on ice for 15 min to facilitate adsorption of the phage to the host cells, then incubated overnight at 37°C. The phage culture was filtered through a 0.2 μm cellulose acetate syringe filter to remove host bacteria and then stored at 4°C. Typical yields were 1×10^8 PFU/mL.

Culturable counts of phage were performed by mixing 100 μL of phage suspension and 100 μL of host culture in 4 mL molten TSB top agar (containing 0.7% agar). The top agar was vortexed gently, then poured on TSB plates. When cooled, the plates were incubated at 37°C.

2.3. Exposure of bacteria and bacteriophage to electric current in an electrochemical cell

The electrochemical cell consisted of a sterile 250 mL beaker and two platinum-tipped copper wire electrodes. The beaker was filled with 10 mL of a conducting medium, EE buffer (30 mM Tris, 150 mM KCl, pH 8.3), and the electrodes tips were held suspended 5 cm apart in the EE buffer with a #13 rubber stopper. Power was supplied to the electrodes with a Hoefer DS500XT DC Power Supply (Hoefer Scientific Instruments, San Francisco, CA).

Bacteria from overnight cultures or bacteriophage from freezer stocks were diluted into EE buffer to a final population density of either approximately 1×10^3 CFU or PFU/mL (low population density), or 1×10^6 CFU or PFU/mL (high population density). Dilutions were prepared in 1X phosphate-buffered saline solution. Initial culturable counts of the bacteria or phage suspension were performed as above in triplicate.

To study the effect of direct current magnitude in the electrochemical cell on viability, 10 mL aliquots of the bacteria or phage suspension were transferred to sterile 250 mL beakers, and pre-exposure culturable counts were performed in triplicate. The electrochemical cell was assembled, and the suspensions were exposed to 5-second bursts of current ranging from 25–350 mA (25–350 V). Post-exposure culturable counts were then performed in triplicate. These counts were divided by the mean unexposed control counts to normalize the data, and then log-transformed. The number of replicates performed for this and subsequent experiments are included in Table 1.

To study the effect of direct current exposure length on viability, two 10 mL aliquots of the bacteria or phage suspension were transferred to sterile 250 mL beakers. The microbe suspension in one beaker was continuously exposed to 5 mA direct current (5 V), as above. At

Table 1
Linear regression statistics for the data depicted in Figs. 1–3

Organism	Population density[a]	GSH (1 mM)	Figure reference[b]	Replicates[c]	Regression statistics				Regression line			
					F	df[d]	P	R^2	Slope ($\log mA^{-1}$)	Slope SE[e]	y-intercept (unitless)	y-intercept SE[d]
MS2	Low	–	1a	3	0.0040	1, 29	0.9499	0.0001	8×10^{-6}	0.0001	0	—
	High	–	1b	3	1.3944	1, 29	0.2473	0.0459	0.0001	0.0001	0	—
	Low	–	2, 3	3	428.2281	1, 20	6×10^{-15}	0.9554	−0.1281	0.0062	0	—
	High	–	2	2	86.6896	1, 13	4×10^{-7}	0.8696	−0.0720	0.0077	0	—
	Low	+	3	2	73.3706	1, 14	1×10^{-6}	0.8398	−0.0435	0.0051	0.1624	0.0701
PRD1	Low	–	1a	3	160.0186	1, 29	2×10^{-13}	0.8466	−0.0008	0.0001	0	—
	High	–	1b	3	80.8861	1, 27	1×10^{-9}	0.7497	−0.0022	0.0002	0	—
E. coli	Low	–	1a	3	36.9111	1, 22	4×10^{-6}	0.6266	−0.0067	0.0011	0	—
	Low	–	2, 3	3	34.1831	1, 11	0.0001	0.7565	−0.2664	0.0456	0	—
	High	–	2	2	72.9062	1, 7	0.0001	0.9124	−0.3073	0.0360	0	—
	Low	+	3	2	43.1747	1, 13	1.8×10^{-5}	0.7688	−0.0576	0.0088	0	—
P. aeruginosa	Low	–	1a	2	108.3626	1, 10	1×10^{-6}	0.9155	−0.0390	0.0037	0	—
	High	–	1b	2	82.2701	1, 13	1×10^{-6}	0.8635	−0.0085	0.0009	0	—

[a] Low population density is approximately 1×10^3 CFU or PFU/mL. High population density is approximately 1×10^6 CFU or PFU/mL.
[b] See the indicated figure and figure legend for a full description of the experimental treatment for each trend.
[c] Indicates the number of trials used to generate each data set and trend line.
[d] df = degrees of freedom.
[e] SE = standard error.

various time intervals, 100 μL samples were taken from the exposed suspension and the unexposed control. Culturable counts were performed on the samples in triplicate. Viability data for this experiment were normalized as above.

2.4. Protective effect of reduced glutathione (GSH)

Additional suspensions of *E. coli* and bacteriophage MS2 were prepared containing 1 mM GSH [16] and exposed to 5 mA over time in conjunction with suspensions without GSH. The viability of the organisms in the presence and absence of GSH for the given treatment were compared.

2.5. Statistics

Analysis of covariance (ANCOVA) and linear regression were performed using Systat statistical software (SPSS Inc., Chicago, IL). Results of all F tests were considered significant at 95% confidence if $P<0.05$ for a given F statistic. If regression analysis indicated that the constant (y-intercept) of the regression line was insignificant, then the regression was performed again with the regression line forced through $y=0$. Intervals representing one standard error in both the y-intercept (if included in the regression) and slope of the regression lines are included in each figure.

2.6. Temperature controls

Ten milliliter aliquots of sterile EE buffer were exposed in the electrochemical cell to a 55 mA direct current for 20 min to test the temperature response of the buffer to the conduction of current. The temperature rose from its initial temperature of 31°C–34°C during the course of the experiment. Temperature was also monitored during the current exposure length experiment, including the longer trials involving GSH treatment. Temperature rose an average of 0.3°C (SD = 0.4°C, $n=13$) over the 20–30 min duration of these trials. It was therefore concluded that the effects of direct current on the viability of bacteria and bacteriophage were not due to thermal inactivation.

3. Results and discussion

ANCOVA indicated that the population of viruses or bacteria varied significantly with the magnitude of applied direct current at low population density ($F=144.4289$, df = 3, 86, $P=2.0109\times10^{-11}$). The population also varied significantly with the type of microbe if current magnitude were covariate ($F=44.5662$, df = 3, 86, $P=1.5206\times10^{-11}$). Specifically, bacteriophage MS2 and PRD1 survived exposures to all magnitudes of direct current in the electrochemical cell at low population density far better than their bacterial counterparts. This is demonstrated by comparison of the least-squares regression line for each data set (Fig. 1a). The regression line for MS2 was not significant (Table 1) due to the fact that the population of MS2 did not vary much with current. But, the MS2 regression line is included in Fig. 1a for comparison with the others. None of the standard error intervals for the regression lines in Fig. 1a overlap, so the regressions for each of the four organisms are statistically distinct from each other. Bacteriophage culturable counts were relatively unaffected by the treatment, but both bacteria populations were reduced by at least one order of magnitude from the pre-exposure culturable counts when exposed to 200 mA or greater.

At high population density, the results differ slightly (Fig. 1b). Like the previous experiment, the variation of population with current was significant ($F=116.8162$, df = 3, 73, $P=2.1006\times10^{-11}$), as was the variation of population with the type of microbe if current is covariate ($F=25.4361$, df = 3, 73, $P=1.7841\times10^{-11}$). The regression for the nearly horizontal MS2 data was not significant (Table 1), but the line is included in Fig. 1b for comparison with the others. The standard error intervals of the regression lines still do not overlap, demonstrating that the data sets for each organism are statistically distinct from each other. The effects of the treatment were again minimal in the case of the bacteriophages. However, the decline of the bacterial population with increasing current was not as severe as at low population density. The slope of the regression line for *P. aeruginosa* decreased by a factor of 4.6 (Table 1). The greater tolerance of *P. aeruginosa* to 5-s pulses of direct current at higher population density suggests a population-dependent protective effect against the mechanism of inactivation of bacteria by direct current.

Despite the better survival of *P. aeruginosa* at higher population density, the bacteriophage still survived the electrochemical treatment better. The slope of the regression line for *P. aeruginosa* was 3.9 times greater than that of PRD1 (the most responsive of the two bacteriophages). The *P. aeruginosa* population was reduced by an order of magnitude when exposed to 150 mA, but PRD1 suffered less than an order of magnitude reduction when exposed to as much as 350 mA.

E. coli and MS2 were then compared for their ability to withstand longer exposures to a 5 mA direct current in the electrochemical cell at low population density (Fig. 2a). MS2 resisted the treatment better than *E. coli*. The ANCOVA results show that population varied significantly with elapsed time of exposure ($F=57.5105$, df = 1, 29, $P=2.3234\times10^{-8}$), as did population with the type of microbe if time were covariate ($F=7.7641$, df = 1, 29, $P=0.0093$). The regression lines for both

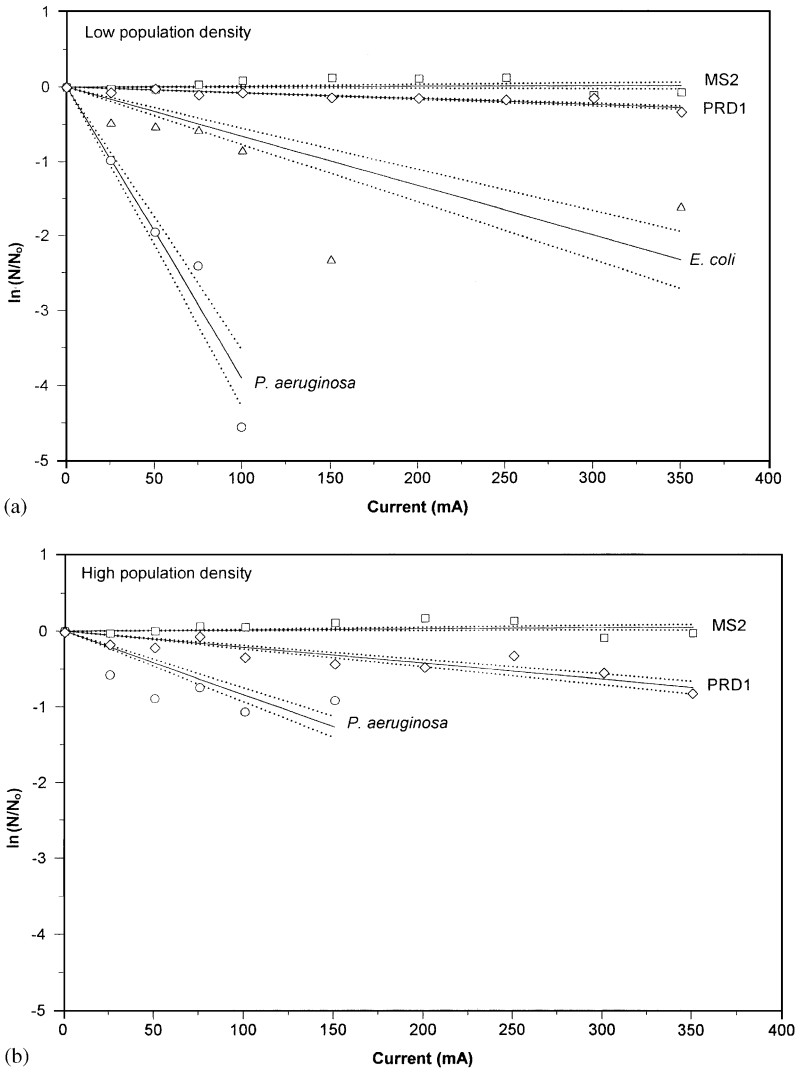

Fig. 1. Effect of direct electric current magnitude upon the viability of the bacteriophages MS2 (□) and PRD1 (◇), and the bacteria *E. coli* (△) and *P. aeruginosa* (○). Suspensions of bacteria or bacteriophage were exposed to various direct current magnitudes for 5 s. Post-treatment culturable counts are reported as a proportion of the pre-treatment culturable counts for each microbial suspension, log transformed. Solid lines indicate the least squares regression lines for the data, with flanking dotted lines indicating the standard error of the regression. (a) Low population density (approximately 1×10^3 PFU or CFU/mL). (b) High population density (approximately 1×10^6 PFU or CFU/mL).

data sets were significant (Table 1), and the standard error intervals around the regression lines for MS2 and *E. coli* did not overlap, confirming that the data sets are statistically distinct from each other. Both MS2 and *E. coli* steadily lost viability during the course of the exposure, though the *E. coli* population declined much faster. In fact, the inactivation rate (slope of the regression line) for *E. coli* was greater than for MS2 by a factor of 2.1 (Table 1). It took only 5 min for the population of *E. coli* to fall beneath the detection limit of the culturable count methodology, whereas MS2 plaques were detectable for the full 20 min.

The results for *E. coli* in this experiment are comparable to similar experiments in the literature. Patermarakis and Fountoukidis [5] exposed total coliforms at population densities of 200–26,800 cells/mL in natural surface water to 2.5 mA/cm^2 and reduced the culturable counts by an order of magnitude in 15.7 min. Matsunaga et al. [4] reduced *E. coli* K12 from a population density of 10^2 cells/cm^3 in water to less than 2% of the original numbers with a 10-min exposure to 0.7 V.

The differences between the *E. coli* and MS2 data were more pronounced at high population density

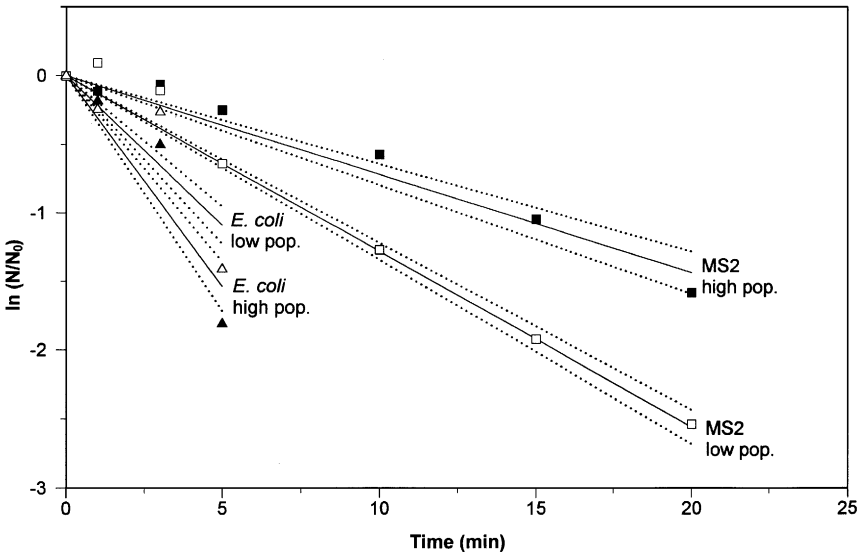

Fig. 2. Effect of the length of exposure to 5 mA direct current upon the viability of bacteriophage MS2 (□) and *E. coli* (△) at both low population density (approximately 1×10^3 PFU or CFU/mL) and high population density (approximately 1×10^6 PFU or CFU/mL). Post-treatment culturable counts are reported as a proportion of the pre-treatment culturable counts for each microbial suspension, log transformed. Solid lines indicate the least-squares regression lines for the data, with flanking dotted lines indicating the standard error of the regression.

(Fig. 2b). ANCOVA indicated that variation in population with time ($F = 54.3960$, df = 1, 18, $P = 1 \times 10^{-6}$) and with the type of microbe if time were covariate ($F = 21.9046$, df = 1, 18, $P = 0.0002$) were significant. The linear regressions for both organisms were statistically significant (Table 1). The standard error intervals around the regression lines for MS2 and *E. coli* did not overlap with each other, so the data sets are statistically distinct from each other. Viable *E. coli* still became undetectable after 5 min, and MS2 plaques were still detectable after 20 min of exposure. According to Table 1, the inactivation rate of *E. coli* was 4.3 times greater than that of MS2 at high population density, compared to 2.1 at the low population density. So, the bacteriophage survived exposure to 5 mA over time better than the bacterium at both high and low population density.

The standard error intervals of the regression lines for *E. coli* at low and high population density overlap, so there is no significant difference in inactivation rate for *E. coli* for differing population densities. This is not the case for MS2. The inactivation rate for MS2 at high population density decreased by a factor of 1.8 relative to low population density (Table 1). This is another example of a protective effect against inactivation at higher population density, but in this case the bacteriophage benefited rather than the bacteria from the effect.

GSH was added to *E. coli* and MS2 suspensions at low population density, which were exposed to 5 mA current over time. The variation in population of the GSH-treated suspensions with the length of current exposure was compared to the response of suspensions without GSH determined in the previous experiments. When GSH was added, it dramatically reduced the ability of the electrochemical cell to inactivate the microbes (Fig. 3). As in the previous experiments, population varied significantly with time for both *E. coli* ($F = 31.3819$, df = 1, 22, $P = 1.2 \times 10^{-5}$) and MS2 ($F = 308.2902$, df = 1, 33, $P = 1.7449 \times 10^{-11}$). Furthermore, treatment (with GSH or without GSH) had a significant effect on population if time were covariate for both *E. coli* ($F = 13.0601$, df = 1, 22, $P = 0.0015$) and MS2 ($F = 82.9299$, df = 1, 33, $P = 1.5980 \times 10^{-10}$). All regressions were significant (Table 1), and the slopes of the regression lines were different between the treatments with GSH and those without. Since the standard error intervals around the regression lines for the GSH-treated versus untreated suspensions did not overlap, the differences in inactivation rate are statistically different. The inactivation rate for *E. coli* decreased by a factor of 4.6 when provided with GSH, and the inactivation rate for MS2 decreased by a factor of 2.9. Since GSH decreased the inactivation rate of the microbes so significantly, the mechanism of inactivation in the electrochemical cell is most likely due to oxidation by electrochemically generated oxidants rather than direct oxidation by electric current or membrane permeabilization (in the case of *E. coli*).

Three significant observations proceed from the results of these experiments. The first is the fact that bacteriophage were able to tolerate greater direct current magnitudes and greater durations of exposure to direct

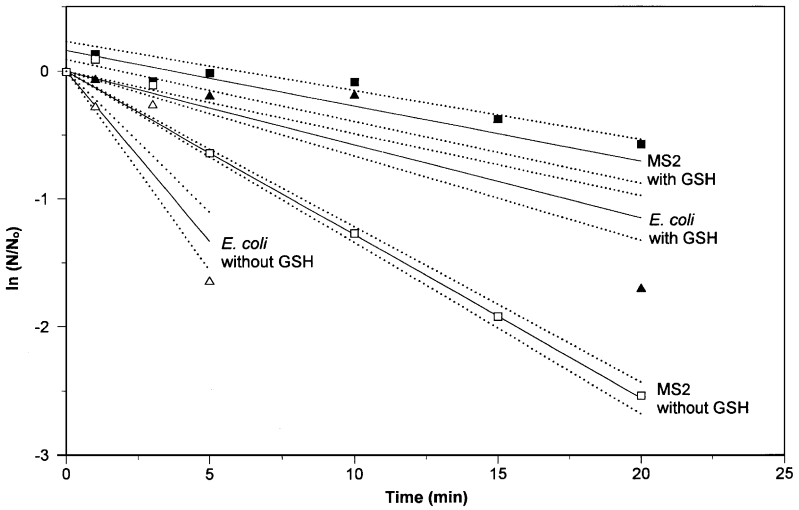

Fig. 3. Protection of *E. coli* and MS2 from inactivation in the electrochemical cell by GSH. *E. coli* cell suspensions (1.23×10^3 CFU/mL) and) MS2 suspensions (5.8×10^2 PFU/mL) were exposed to 5 mA for 30 min. Post-treatment culturable counts are reported as a proportion of the pre-treatment culturable counts for each microbial suspension, log transformed. Solid lines indicate the least squares regression lines for the data, with flanking dotted lines indicating the standard error of the regression. (△) *E.* coli, 0 mM GSH, (▲) *E.* coli, 1 mM GSH, (□) MS2, 0 mM GSH, (■) MS2, 1 mM GSH.

current in the electrochemical cell than bacteria. This is the first study in which the electrochemical inactivation of viruses has been examined. Technologies implementing electrochemical inactivation of bacteria have been suggested for applications that affect human health, such as the pasteurization of food [3] and treatment of drinking water [4,5]. But, pathogenic human enteric viruses, including the rotaviruses, astroviruses, enteroviruses, Norwalk-like viruses, adenoviruses, and hepatitis A virus, may be found in wastewater and contaminated drinking water sources [14]. Food crops irrigated with wastewater may be contaminated with these pathogens as well [14]. This study indicates electrochemical treatments designed to reduce the numbers of bacteria in food and water may not be sufficient to kill viruses, and therefore the safety of treated water and food cannot be guaranteed unless this is addressed.

Like MS2 and PRD1, the enteric viruses are non-enveloped and encapsulated with a protein capsid [18]. The similarities between the bacteriophages tested in these experiments and the enteric viruses suggest that these pathogens may be expected to tolerate exposure to electric current much like MS2 and PRD1. The minimum infectious dose (MID) of pathogenic enteroviruses is also generally much lower than the MID of bacterial pathogens [14]. Direct current or electric field magnitudes and treatment times must be sufficient to reduce viruses, not just bacteria, below MID before treated food and water can be considered safe.

The second finding of this study is that extracellular GSH moderated the inactivation of both *E. coli* and MS2 when exposed to 5 mA over time in the electrochemical cell. GSH acts by reducing the oxidants produced in the electrochemical cell before they have a chance to damage the cells or viral particles. GSH can be oxidized directly by the electric field within the electrochemical cell, but this cannot prevent the same field from inactivating the microorganisms present in cell. GSH also cannot protect cells from membrane permeabilization by the electric field in the cell. Thus, the significant influence of GSH on the inactivation of the microbes confirms that the inactivation is primarily due to the disinfectant properties of electrochemically generated oxidants produced at the electrodes in the cell.

Finally, there is a population-dependent effect upon the efficacy of the electrochemical cell in inactivating bacteria and bacteriophage. This protective effect was observed for *P. aeruginosa* in the current magnitude experiment (Fig. 1), which shows clearly that the bacteria were capable of tolerating greater magnitudes of direct current at higher population density. The protective effect was also observed for bacteriophage MS2 in the exposure length experiment (Fig. 2). Matsunaga et al. witnessed a similar effect in their electrochemical reactor [4]. Beginning with 10^2 cells/cm^3, the surviving fraction of cells after a 10 min 0.7 V electrochemical treatment was less than 2%. But beginning with a population density of 10^4 cells/cm^3, the fraction of surviving cells rose to approximately 25%. Patermarakis and Fountoukidis did not report this effect, however [5]. Sterilization of water with their electrochemical cells was accomplished after a 15.7 min

exposure to 2.5 mA/cm^2 (125 mA) regardless of the initial population density.

This population-dependent effect can be explained by the fact that inactivation of microorganisms by electrochemically generated oxidants is the major mechanism at work in the electrochemical cell. During an experiment, a finite amount of oxidants are produced at the electrodes. These oxidants can inactivate a greater proportion of the microorganism population when the population is low compared to when the population is high.

The population-dependent protective effect experienced by the bacteria may also be due to the production of catalase. *P. aeruginosa* is an aerobic bacterium, and as such, is capable of producing the enzyme catalase. The high population density suspensions would presumably contain more catalase than the low population density suspensions, which would offer more protection to the cells from the activity of hydrogen peroxide produced at the electrodes. This is speculation, however; the concentration of catalase in the bacterial suspensions was not measured in these experiments. Note however that *E.* coli, which is capable of producing catalase, did not demonstrate this protective effect as expected. It should be emphasized that viruses cannot produce catalase, and despite this lack of a potential protection mechanism, bacteriophage still demonstrated greater resistance to inactivation in the electrochemical cell than bacteria.

The population-dependent protective effect has implications for application of electrochemical inactivation. Protocols capable of inactivating a low number of microorganisms may be ineffective when challenged with more highly contaminated samples, so electrochemical inactivation techniques must be optimized for expected levels of contamination.

4. Conclusions

Bacteriophages survived short exposures to various current magnitudes in an electrochemical cell better than bacteria at both low (1×10^3 CFU or PFU/mL) and high (1×10^6 CFU or PFU/mL) population density.

The inactivation rate of bacteria exposed to a low current magnitude (5 mA) for an extended time ranged from 2.1 to 4.3 times greater than that of bacteriophages, demonstrating that bacteria are more sensitive to electrochemical inactivation than bacteriophages.

Reduced glutathione (GSH) reduced the inactivation rate of microbes in the electrochemical cell by a factor of 2.9–4.6. This indicates that electrochemically generated oxidants were a major cause of inactivation within the electrochemical cell.

The electrochemical cell was more effective at inactivating microbes at lower population densities than at higher. This may be attributed to titration of the electrochemically generated oxidants, and perhaps also the greater production of catalase by bacteria at higher population densities.

Since bacteriophages are more resistant than bacteria to electrochemical inactivation, use of this technology in fields that affect human health (such as drinking water disinfection) must ensure the destruction of viruses, not just bacteria, in order to consider the treated medium safe.

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