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Chlorine-free Electrochemical Disinfection of Water Contaminated with Salmonella Typhimurium and E.Coli B

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Abstract

Deionized (DI) water contaminated with Salmonella Typhimurium (S. Typhimurium) or E.Coli B bacteria was disinfected by alternating current. Ammonium sulfate, sodium nitrate and phosphate buffer were used as electrolytes. Disinfection was carried out in the circulation system including an electrochemical cell with three types of electrodes; stainless steel, titanium and nickel. The process efficiency was estimated and the number of killed bacteria was directly proportional to the water treatment time and concentration of hydroxyl radicals generated by electrolysis. The presence of OH radicals was detected with N,N-dimethyl-p-nitrosoaniline (RNO) used as a spin trap. Similar experiments were carried out with water remaining after poultry washing at poultry farms and additionally contaminated with S. Typhimurium bacteria. Measures were recommended

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to increase the process efficiency and decrease the water treatment time.

Introduction

Sewage water contains numerous microorganisms. There are numerous methods to reduce the number of infection components to an acceptable level for drainage to any water pools (1). Earlier, the chlorination method was traditionally used; in recent years, ozone treatment, ultraviolet irradiation, and various electrochemical processes are applied. Selection of the disinfection method significantly depends upon the cost of required energy, especially in the cases, where large quantities of water should be treated.

S. Typhimurium and E.coli bacteria are classified as the most dangerous microorganisms causing serious intestinal disorders of humans. Food products of animal and vegetable origin are often infected with both types of bacteria. Water remaining after poultry washing at poultry farms (rinsing water) is also often infected with these bacteria. Recently, poultry water was disinfected by such chemical methods as chlorination (2), ozonation (3), and others (4). The disadvantages of these methods include potential formation and accumulation of toxic chemical products as, for example, in the case of chlorination or high cost as in the case of ozonation (5).

Electrochemical treatment of water, including rinsing water became interesting relatively recently (1, 6–12). The authors of study (1) found that effective electrolytic water treatment using NaCl electrolyte is accompanied by generating significant quantities of elementary chlorine, hypochlorite, and, probably, chlorate and requires a high initial concentration of NaCl (over 0.15%). At such concentration, chloride anions cause corrosion of any bimetal parts of water systems, for example, water distributors, cooling towers, etc. Replacement of chloride anions with phosphates, nitrates, sulfates, or carbonates, which do not cause corrosion, is more favorable with respect to the equipment operation costs and increases profitability of the electrochemical water sterilization process.

The mechanism of electrochemical disinfection depends upon numerous parameters, including the nature of electrolyte. Calculations of the

number of *S. Typhimurium* bacteria in NaCl, Na₂SO₄, and Na₃PO₄ solutions subjected to low voltage and low current electrolysis were performed (6, 13, 14). Already in five minutes, this treatment was shown to reduce the concen tration of live *S. Typhimurium* bacteria by one or two orders of magnitude.

The authors of (15-17) studied the electrochemical disinfection mechanism for *E. coli* B bacteria in NaCl, NaNO₃, and Na₂SO₄ solutions. The disinfection efficiency of electrolysis was close to that of ozonation, i.e., much higher than that of ordinary chlorination. Electrochemical disinfection was shown to kill bacteria not by electric field. In the opinion of the authors of (18), the major contribution to the disinfection effect is made by generation of hydroxyl radicals. The formation mechanism of OH radicals in electrolysis of aqueous solutions was discussed in studies (19-21). They authors showed, in particular, that the generally accepted schemes of anodic oxidation and cathodic reduction are simplified for the reactions:

$$2H_2O = 4H^+ + 4e + O_2$$

 $4H_2O + 4e = 2H_2 + 4OH^-$

Formation of atomic hydrogen (H*) is one of the intermediate stages in the course of molecular hydrogen formation in the reaction of cathodic reduction. Here a part of atomic hydrogen initiates the following sequence of reactions:

$$H^* + O_2 = HO_2$$

 $HO_2 + H^* = H_2O_2$

In the cases, where electrodes are made of metal (M), hydrogen peroxide takes part in the reaction generating hydroxyl radicals:

$$H_2O_2 + M^{2+} = M^{3+} + \bullet OH + OH^-$$

The life time of hydroxyl radicals is too short for their identification by ESR method. Nevertheless, an effective method for detection of hydroxyl radicals was suggested by the authors of (21). They analyzed the interaction products of hydroxyl radicals with *tert*-butylnitron in electrolysis of potassium chlorate solutions. The generated long-living radicals were detected by ESR method.

In addition to hydroxyl radicals, the disinfection process may involve also other intermediate highly active compounds such as O_2 anion radicals. In the case of sulfate electrolyte (22), there may be persulfate and sulfate radicals. In electrolysis of phosphate buffer (23, 24), bacteria are killed due to formation of hydrogen peroxide.

Our earlier studies represent the results from electrochemical disinfection of deionized (DI) water contaminated with *E. coli B* (25) and *S. Typhimurium* (26) containing (NH₄)₂SO₄ as electrolyte. We suggested a kinetic model for elimination of bacteria and a quantitative method describing the rate of interaction between hydroxyl radicals and bacteria and considered the effects of such parameters as treatment time, electrolyte concentration, and initial concentration of bacteria. In this work, we analyzed the results of a similar study carried out for DI water and poultry water containing three different types of electrolytes contaminated with a high concentration of *S. Typhimurium* and *E. coli B* bacteria.

Experimental

We used N,N-dimethyl-p-nitrosoaniline (RNO) which is known (15) as a spin trap for hydroxyl radicals. The concentration of OH radicals resulting from electrolysis was estimated with a spectrophotometer by the change in the RNO optical absorption spectral intensity with the peak at 440 nm. The solutions of DI water contained the (NH₄)₂SO₄ NaNO₃ or phosphate buffer (NaH₂PO₄ + Na₂HPO₄) electrolyte concentration of 0.025 to 0.5% and RNO concentration of 0.0027 to 0.003% (1.8 × 10^{-6} to 2.0×10^{-6} M). The poultry rinsing water was provided by the experimental poultry plant at the University of Arkansas (Fayetteville, Arkansas). Before electrolysis, this water was diluted with the same volume of DI water containing the necessary quantities of electrolyte and RNO. According to the results of microbiological analysis, the population of S. Typhimurium bacteria in the prepared solution was below the detection level. We prepared the solutions of S. Typhimurium bacteria with various concentrations by dilution of water with highly concentrated suspension containing approximately $42 \times 10^{\circ}$ cfu/ml of bacteria.

Our experimental water disinfection device is represented in Figure 1. We found that alternating current of 0.21 A (50 Hz frequency) corresponding to the current density of 60 mA/cm2 does not cause corrosion of steel electrodes in the selected range of electrolyte concentrations.

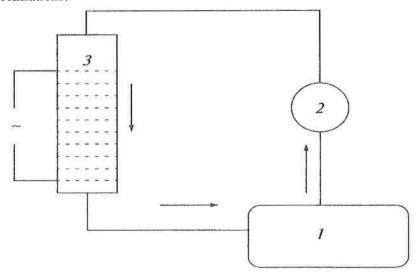


Figure 1. Laboratory electrochemical water disinfection device: (1) pump, (2) water flow meter, (3) plastic electrochemical cell; parallel set of ten stainless steel net electrodes (5.5 cm diameter) with 3 mm intervals. (Reproduced with permission from reference 26. Copyright 2010 Pleiades Publishing, Ltd)

We maintained the constant current density by selecting the initial voltage in the range from 40 to 170 V with respect to the electrolyte concentration. Before starting each experiment, the laboratory device was disinfected for 20 min with hot water flow at the temperature of 75 to 80°C. The initial temperature of the treated water was 20°C. The water flow rate through the electrochemical cell was 10 Gallon/min. The portions of 10 ml were sampled through the open cover of electrochemical cell and stored at 4°C for microbiological analysis. The colonies of *S. Typhimurium* and *E. coli B* were counted after incubation for 48 h at 35°C.

Results and Discussion

Figure 2 represents a typical curve of live bacteria concentration in solution vs. electric current treatment time. Our analysis of experimental data enables to make to conclusions. First, without electric current, no addition of electrolyte and RNO to DI water contaminated with S. Typhimurium causes any significant bactericidal effect. Second, if electric current is applied, the number of live bacteria vs. treatment time can be described with a linear logarithmic curve. To describe the process of electrolysis, we used a modified kinetic model designed for the disinfection of natural water contaminated with coliforms (15).

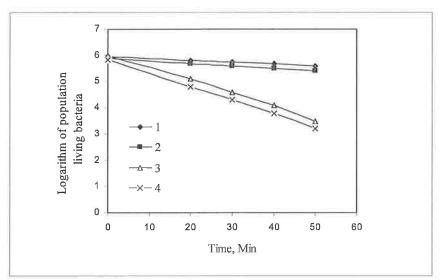


Figure 2. Live *S. Typhimurium* bacteria count vs. disinfection time in $(NH_4)_2SO_4$ solutions with concentrations of 0.025% (1, 3) and 0.05% (2, 4) without electrolysis (1, 3) and with electrolysis (3, 4).

According to this model, the initial number of bacteria (n_0) and the current number of bacteria (n) at time moment t are interrelated as follows:

$$Log n = log n_0 - kt \tag{1}$$

were k is the factor depending on the current density for the constant volume of water to be disinfected and constant surface area of the electrodes.

The diagram in Figure 2 enables to calculate factor k and to determine such essential parameter of disinfection process as the minimum time $t_{\rm d}$, which is necessary to decrease the concentration of bacteria to n=1. With respect to calculation of the bacteria population in the selected dimension, such low concentration should be considered as negligibly small. Therefore, $t_{\rm d}$ can be referred to as the time required for complete elimination of bacteria. It corresponds to the intersection point of the experimental line with the time axis. Table I represents the parameters of k and $t_{\rm d}$ calculated for each concentration of electrolyte.

The contaminated water flow through the electrochemical cell without the applied voltage slightly decreases the population of bacteria, which becomes noticeable after 30 min experiment. A similar effect was earlier noted for DI water contaminated with $E.\ coli\ B\ (26)$ and seems to be accounted for by the known biocide effect of cavitation to microorganisms.

Our electrochemical experiments with poultry rinsing water contaminated with S. Typhimurium bacteria were carried out under the same conditions as our experiments with DI water. First, as in the case of DI water, electrochemical disinfection of rinsing water takes place far more effectively than water flow through electrodes under no voltage. Second, the live bacteria count vs. water treatment time is described with logarithmic curve. Table I shows a comparison between factor k and time t_d for poultry water with the corresponding data for DI water. One can see that achievement of complete disinfection by electrochemical treatment of S. Typhimurium bacteria in poultry water requires more time than in the case of DI water.

Generation of OH radicals by electrochemical treatment of contaminated water was successfully used for oxidation of organic compounds, for example, phenols (28). The advantages of RNO used as a spin trap are accounted for by high selectivity of the reaction of interaction between RNO and OH radicals. Consider the reactions of OH radicals spending in the course of electrolysis. The interaction scheme can be represented as follows:

•OH + spin trap \rightarrow spin adduct

Figure 3 shows the change in the absorption spectra of RNO in $(NH_4)_2SO_4$ aqueous solution contaminated with *S. Typhimurium* under the conditions of electrolysis.

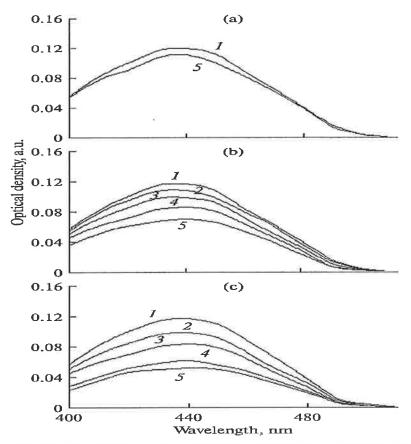


Figure 3. Absorption spectra of RNO in 0.2% solution of $(NH_4)_2SO_4$ in DI water contaminated with *S. Typhimurium* before (1) and after flow through electrochemical cell for 5 (2), 20 (3), 40 (4), 60 (5) min; water treated without voltage (a), under voltage (b), under voltage in bacteria free solution (c). (Reproduced with permission from reference 26. Copyright 2010 Pleiades Publishing, Ltd)

Table I. Factor k and complete disinfection time t_d , calculated for linear model (equation 1) for electrolysis of aqueous S. Typhimurium solutions (Reproduced with permission from reference 26. Copyright 2010 Pleiades Publishing, Ltd).

Concentration of (NH ₄) ₂ SO ₄ , %	Disinfection media	Log n _o [cfu/ml]	k	t _d ,
0.025	DI water	6.01	0.0499	120.2
	Poultry water	5.76	0.0448	128.6
0.05	DI water	6.06	0.0550	110.1
	Poultry water	5.68	0.0464	122.3
0.2	DI water	6.13	0.0565	108.5
	Poultry water	5.63	0.0472	119.2
0.5	DI water	6.21	0.0634	98.0
	Poultry water	5.59	0.0503	111.2

As one can see, a solution flow through the zero current electrolytic cell only slightly decreases the optical density at 440 nm (Figure 3). Under the conditions of electrolysis, the optical density changes rapidly. It decreases far more rapidly if electrolysis takes place in the bacteria free solution. These results apparently evidence formation of OH radicals.

One can neglect potential occurrence of other reactions involving hydroxyl radicals, excepting elimination of bacteria and interaction with RNO. Then expenditure of OH radicals in electrochemical treatment can be described as follows (26):

'OH + RNO
$$\xrightarrow{k_{OH}}$$
 R-(HO)NO',
'OH + E. coli B $\xrightarrow{k'_{OH}}$ dead bacteria.

We use the kinetic model suggested for description of two competing reactions (28):

$$1/G_t = 1/G_o x \{ 1 + (k'_{OH} [B]) / (k_{OH} [RNO]) \}$$
 (2)

where G_l is the bleaching rate of RNO in the presence of bacteria; G_o is the bleaching rate of RNO in the absence of bacteria; [B] is the bacteria concentration in the electrolyte; [RNO] is the concentration of RNO in the electrolyte; k'_{OH} and k_{OH} are the rate constants of the corresponding reactions.

Like in the case of DI water, voltage applied to poultry rinsing water sharply decreases the RNO optical density at 440 nm. Compare the kinetic characteristics of RNO interaction with hydroxyl radicals in DI water and poultry water. For this calculation, we selected the (NH₄)₂SO₄ concentration of 0.2% and various initial concentrations of *S. Typhimurium* (Table II).

The RNO concentration was measured after 40 min treatment of water. The $1/G_t$ vs. [B]/[RNO] is a linear relationship with the slope of

 $I/Go(k'_{OH}/k_{OH})$. The radical-bacteria interaction rate constant was calculated on the assumption the RNO-OH radical reaction rate constant is $1.2 \times 10^{10} \, \mathrm{mol}^{-1} \, \mathrm{s}^{-1}$ (4). Thus calculated values of k'_{OH} for DI water and poultry water are represented in Table 2 along with the values

of constant k and time t_d for both kinds of water as calculated by equation (1). One can see that the bacteria elimination rate in DI water is higher than that in poultry water.

Table II. Factor k and complete disinfection time t_d , calculated by equation (1) and S. Typhimurium elimination rate constants calculated by equation (2) (Reproduced with permission from reference 26. Copyright 2010 Pleiades Publishing, Ltd).

Disinfection	Log no	k	$t_{d,}$	k' _{OH}
media	[cfu/ml]		min	[cfu/sec]
DI water	6.13	0.057	109	3.9×10^6
DI water	6.32	0.055	116	
DI water	6.43	0.053	122	
DI water	6.85	0.055	125	
Poultry water	5.63	0.047	119	1.7×10^6
Poultry water	5.76	0.047	124	
Poultry water	5.89	0.046	127	
Poultry water	6.05	0.046	130	

Therefore, disinfection of poultry water is a slower process than disinfection of DI water. This conclusion follows from comparison of the bacteria elimination rate and agrees with the determined time $t_{\rm d}$ required for complete disinfection of water. As follows from (29), the bacteria elimination rate under electrochemical treatment of S. Typhimurium suspensions is higher in DI water than that in poultry water. This difference was accounted for by the fact that poultry water contains additionally blood cells, fat micelles, and other organic substances, which can coat the cells of bacteria and thus protect them from the effect of radicals. Such explanation seems to be true also for interpretation of the results represented in this work.

Table III represents the results from electrochemical treatment of DI water contaminated with bacteria of two species. All the measurements were carried out with the same electrolyte in the same concentration under the same conditions of AC treatment. Apparently, for approximately the same initial concentration of bacteria (deviation not exceeding 3%), complete disinfection in the case of *E. coli B* needs less time than in the case of *S. Typhimurium*.

Our conclusion that disinfection of *S. Typhimurium* is a slower process as compared to that of *E. coli B* is confirmed with the bacteria elimination rate constants k'_{OH} . These constants were calculated in (26)as 3.91×10^6 and 6.01×10^6 cfu/s, respectively.

The efficiency of electrochemical disinfection can be enhanced also by varying such parameters as water volume and flow rate through electrochemical cell as well by changing the nature of the electrode materials. Data presented in Figure 4 and Table IV are obtained during electrolysis with two different types of electrodes for DI water containing two different electrolytes at concentration 0.5% contaminated with *S. Typhimurium*.

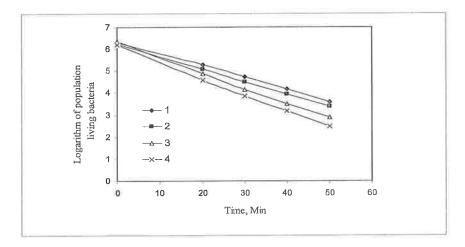


Figure 4. Live *S. Typhimurium* bacteria count *vs.* disinfection time in ammonium sulfate solution (1,3) and phosphate buffer solution (2,4)

with two different types of electrodes: titanium (1, 2) and stainless steel

Table III. Complete water disinfection time t_d for various initial concentrations of S. Typhimurium and E. coli B bacteria (stainless steel electrodes are used)

Nature of	Nature of bacteria	$Log n_o$	k	t _d ,
electrolyte		[cfu/ml]		min
Ammonium sulfate	E. coli B	6.23	0.0698	89.3
	S. Typhimurium	6.21	0.0634	98.0
Sodium nitrate	E. coli B	6.19	0.0674	91.9
	S. Typhimurium	6.34	0.0626	101.3
Phosphate	E. coli B	6.16	0.0727	84.7
buffer	S. Typhimurium	6.28	0.0706	88.9

It was found that the AC electrolysis of phosphate buffer solution leads to formation of hydrogen peroxide, which was confirmed by using Hochanadel's colorimetric method (30). According to this method, the concentratuion of hydrogen peroxide is proportional to absorbance at 350 nm related to presence of anions I₃ in product of interaction between H_2O_2 , KI, NaOH, KHC₈ H_4O_4 and (NH₄)₆ Mo_7O_{24} x4 H_2O . It is known that hydrogen peroxide can easily react with ferrous ions giving very active hydroxyl radicals (Fenton reaction): $\mathbf{H_2O_2} + \mathbf{Fe}^{2+} = \mathbf{Fe}^{3+} + \bullet \mathbf{OH} + \mathbf{OH}^-$

$$H_2O_2 + Fe^{2+} = Fe^{3+} + \bullet OH + OH$$

The experimental confirmation of this hypothesis was obtained in following test. The low concentration of ferrous sulfate (0.05%) was added to phosphate buffer during electrolysis of aqueous S. Typhimurium solution with titanium electrodes: complete water disinfection time t_d in this case was decreased from 105.1 min (the same system, but with no ferrous sulfate addition) to 89.3 min.

Table IV. Complete water disinfection time t_d for various initial concentrations of *S. Typhimurium* with three different types of electrodes

Material of electrodes	Nature of electrolyte	Log n _o	k	t _d ,
	(concentration 0.5%)	[cfu/ml]		min
Stainless	$(NH_4)_2SO_4$	6.21	0.0634	98.0
steel	Phosphate buffer	6.28	0.0706	88.9
Titanium	(NH ₄) ₂ SO ₄	6.30	0.0587	107.3
	Phosphate buffer	6.27	0.0596	105.1
Nickel	(NH ₄) ₂ SO ₄	6.38	0.0589	108.3
	Phosphate buffer	6.40	0.0599	106.8

The energy loss of this process decrease at a lower electric resistance of the electrolyte solution, for example, for a shorter distance between electrodes, larger surface area, or higher electrolyte concentration. The process cost can be essentially decreased by selecting the electrolyte, for example, if $(NH_4)_2SO_4$ is replaced with less expensive Na_2SO_4 .

Conclusions

- (1) Chlorine-free AC electrochemical disinfection with chloride-free electrolyte such as ammonium sulfate, sodium nitrate and phosphate buffer is an effective method for disinfection of DI water contaminated with *S. Typhimurium* and *E. coli B* bacteria.
- (2) If all other conditions are the same, disinfection of *S. Typhimurium* in poultry rinsing water requires more time than disinfection of *S. Typhimurium* of DI water.

- (3) If all other conditions are the same, disinfection of *S. Typhimurium* of DI water in electrolytic cell with stainless steel electrodes is more effective than disinfection in electrolytic cell with titanium or nickel electrodes.
- (4) Use of N,N-dimethyl-p-nitrosoaniline as a spin trap experimentally confirmed formation of hydroxyl radicals in electrolysis of aqueous ammonium sulfate solutions. Hydroxyl radicals make an essential impact to the bactericidal process.
- (5) Under the same electrolytic conditions, disinfection of DI water contaminated with *S. Typhimurium* bacteria takes more time than in the case of DI water contaminated with *E. coli B*. This conclusion is confirmed with the kinetic calculations for expenditure of hydroxyl radicals.

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