DOI: 10.55522/jmpas.V11I6.4005 ISSN NO. 2320–7418

International peer reviewed open access journal

Journal of Medical Pharmaceutical and Allied Sciences

Journal homepage: www.jmpas.com CODEN: JMPACO

Research article

Antimicrobial activity and safety applications of electrolyzed water as a microbial disinfectant

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ABSTRACT

One of the promising new disinfectant agents that has recently been suggested as a replacement for traditional decontamination techniques like heat and chemical sanitizers is electrolyzed water. The purpose of this study was to investigate different characteristics of electrolyzed water in order to provide safety and to develop proper sanitation practical guidelines. The tests are carried out by evaluating the chemical, microbiological, and cytotoxicity properties of electrolyzed water. Furthermore, the potency of these disinfectants' sterilization techniques (soaking and spraying) was compared. The findings demonstrate that the electrolyte water has alkaline properties and a shelf life of 7 days. After 1-3 minutes of contact, electrolyzed water has the ability to kill all types of microbes, including bacteria and fungi. The electrolyte water toxicity test and phenol coefficient revealed that it has a low toxicity level and a phenol coefficient of 1-4 to indicator bacteria. Furthermore, immersion techniques up to 3-5 minutes were found to be more effective than spray techniques for surface and/or object disinfection. In conclusion, electrolyte water is impactful at disinfecting surfaces of objects and fabrics while also being safe for cleaning surfaces, clothing, and other equipment. This experiment leads to enhanced safety confidence and serves as a guideline for future better and more appropriate hygiene.

Keywords: Disinfectant, Electrolyzed Water, Anti-Microbial, Cytotoxicity, Chemical Physical Property

Received - 11-07-2022, Accepted- 25-11-2022

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INTRODUCTION

Infectious diseases caused by pathogenic microorganisms kill more people than any other single cause globally. Infections caused by pathogenic microorganisms are a major cause of concern in a variety of fields. There is currently a viral and bacterial disease outbreak. Many recommended procedures were invented in assessing the potential for reducing infectious disease transmission through hygiene practice, such as hand hygiene, contact surfaces should be barrier protected or cleaned and disinfected. Standard chemical solutions, such as sodium hypochlorite, chlorine dioxide, hydrogen peroxide, and organic acids, on the other hand, were irritating to the skin and dangerous to use. As a consequence, discovering alternative disinfectants to replace the old ones is essential.

Electrolyzed water is a clean technology that has recently gained popularity. The technology is based on the electrolysis of sodium chloride-containing water in an electrolysis chamber with anode and cathode electrodes separated by an ion permeable diaphragm. Its principle base on production of hypochlorous substances that are more effective than hypochlorite ions (OCl^-) obtained by dissociation from sodium hypochlorite and calcium hypochlorite ($Ca(OC)_2$) [1, 2]. There were two types at the time: high alkalinity (pH > 11) with low oxidation-reduction potential (ORP 800 mV) and high acidity (pH 2.5) with high ORP value (ORP > 1100 mV) and free chlorine concentration (FCC). Electrolyte water can now be easily produced. Its disinfectant properties were non-toxic, stable, cost-effective, low-cost, and user-safe [3].

The purpose of the study was to search into the chemical properties and stability of electrolyzed water. The bactericidal ability, cytotoxicity, phenol coefficient, and optimal disinfection technique (immersion and spray) of these disinfectants on different surface objects were therefore determined to ensure safety and to further proper hygiene practical guidelines under simulated appropriate in vitro laboratory conditions.

MATERIALS AND METHODS

Microorganisms and chemicals

Ten pathogenic bacteria and 2 pathogenic yeast used in vitro to test the electrolyzed water. The microbial pathogen used in this experiment was helpfully provided by the faculty of Medical technology, Rangsit University. To achieve log phase, the tested microbial strains were re-cultured on agar medium (Tryptic Soy Agar; TSA/Potato dextrose Agar; PDA), and incubated for 24 hours

at 37°C for bacteria and Candida albicans, and room temp., 48 hours for Cryptococcus neoformans. The absorbance from each isolated pure colony was measured and adjusted to the 0.5 McFarland Standard (~1.5 x 10^8 colony forming units per milliliter (CFU/ml)) in sterile 0.85 % NaCl [4]. The 3-(4,5-dimethylthiazol-2-yl)-5-diphenyltetrazolium bromide (MTT), fetal bovine serum (FBS), phosphate buffered saline (PBS), Dulbecco's modified Eagle's medium (DMEM), and trypsin were delivered by GIBCO BRL, Paisley, UK and Thermo Scientific HyClone respectively. The remaining basic reagents were all of analytical grade.

Preparation of electrolyte water

One gram of sodium chloride was dissolved in one liter of distilled water (pH 7.0; 0.1 % w/v) to make electrolyte water. The mixture was imported to produce electrolyte water for 5 minutes using an electrolyte water producing equipment (Alpha-Health and Beauty (Thailand) Co., Ltd.) and was termed "Electrolyte water." The solution was to use it right away or store it in a sealed container at room temperature.

Chemistry property of electrolyte water

The pH and chemical properties were determined using the Suntex TS-100 Suntex Company, USA). Free chlorine and total chlorine were measured using Photometer (Macherey-Nagel, Germany). Chemical stability was tested using the pH and ORP values of oxidized water from day 1 to day 7 after production. Distilled water and 0.6% sodium hypochlorite (NaOCl) were used as negative and standard disinfectant controls, respectively, when comparing chemical properties with electrolyte water.

Determination of bactericidal activity by spread plate technique after direct exposure to electrolyte water

To determine bactericidal activity, 5 mL of tested pathogenic microbial suspension (~ 1.5 x 10^4 CFU/ml for bacteria and ~ 1.5 x 10^3 CFU/ml for yeast) was mixed with 5 mL of electrolyte water. At different time points (30 sec, 1 min, 3 min, 5 min, 10 min and 15 min), 100 µL of the suspension was spread on TSA/PDA plates and incubated for 24 hours at 37°C for bacteria and Candida albicans and 48 hours at room temperature for Cryptococcus neoformans. Following the incubation, colony counts were performed manually in comparison to the control cultures (0.6% NaOCl and sterile NSS as a positive control and growth control) and indicated as colony forming units (CFUs). Individual experiments were replicated three times.

Measurement of intracellular protein leaking by dye-binding method (Bradford)

Intracellular protein leaking by electrolyte water was performed by 5 mL of tested pathogenic microbial suspension (~ 1.5 x 10^4 CFU/ml for bacteria and ~ 1.5 x 10^3 CFU/ml for yeast) was mixed with 5 mL of electrolyte water. After interval time (30 sec, 1 min, 3 min, 5 min, 10 min and 15 min), 10 µL of suspension was mixed with 200 L of Coomassie dye, and the color produced by the Coomassie dye-protein interaction was measured using the dye-binding Bradford method [5]. The optical density (OD) was measured at a wavelength of 595 nm. The protein concentration was calculated using the calibration curve of the bovine serum albumin protein standard curve (BSA). The results were presented in µg of microbial protein/mL and compared to control cultures (0.6% NaOCl and sterile NSS as a positive control and growth control). The three replicates of individual experiment were performed.

Phenol coefficient test of electrolyte water

The phenol coefficient was applying to test the electrolyte water according to previous report [6]. In brief, a phenol stock solution (5 % w/v) was prepared. Then, serial two- fold dilutions of phenol or electrolyte water by sterile distilled water were conducted, yielding concentrations ranging from 5-0.3125 % w/v and 1:2-1:64, respectively. An isolated colony of each indicator microorganisms isolate (Bacillus subtilis / Salmonella typhi) was suspended in a sterile 0.85% NaCl solution, and the turbidity was adjusted equivalent to 0.5 McFarland standards corresponding to 1.5×10^8 CFU/ml and subsequently diluted to 10^4cell/ml. To determine the phenol coefficient, 700 µl of each tested microorganism suspension was added to each tube of serial dilutions of phenol or electrolyte water respectively in the 1:1 ratio. Following the 5- and 10-minute intervals, 100 µl of culture from various dilutions was spread onto TSA and incubated for 24 hours at 37°C. After an incubation period, the bacterium growth was observed, and the Rideal-Walker Coefficient was calculated by dividing the highest dilution of the disinfectant that killed in 5 minutes but not in 10 minutes.

Cell lines and culture medium

Normal human fibroblast (OUMF fibroblast cell lines) stock cells were maintained as monolayer cultures in DMEM supplemented with 10% inactivated FBS, 1% Antibiotic – antimycotic, and 1% Glutamine, in a 5% CO_2 humidified atmosphere incubator at 37°C until confluent. The stock cultures were grown in 25 cm^2 culture flasks, and the cells were dissociated using trypsin–EDTA (0.2% trypsin, 0.02% EDTA in PBS) from their culture flasks twice weekly. All experiments were carried out in 96 microtiter plates (Nunc. Ltd., USA).

Cytotoxicity tests

For preparation of test solutions, electrolyte water was serial concentrations such as 0.1, 1, 10, 25, 50, 75, and 100% v/v was made up with non-supplemented DMEM and sterilized by filtration. The serially dilution were prepared for carrying out cytotoxic studies. The MTT assay was performed as described by Cardile and co-worker, 2004 [7]. The viability of the cell was assessed by MTT assay, which is based on the reduction of MTT by the mitochondrial dehydrogenase of intact cells to a purple formazan product. Briefly,

each cell line (5 × 10^4 cells/well in 100 µl medium) were seeded onto 96-well microtiter plates and routinely cultured in a humidified incubator at 37°C in 5% CO_2 for 24 h. The cultivated cells were separately treated with various serially electrolyte dilution (0.1 -100% v/v) and OMUF cell line cultured in DMEM + 10% heat inactivated FBS was used as growth control. The plate was reincubated for 24 h. Then, 10 µl of MTT dye solution (3-[4,5 -dimethylthiazol-2-yl]-2,5 -diphenyltetrazolium bromide) (5 mg/ml in PBS) was added to every well and reincubated for 4 h. After removing un-transformed MTT reagent, 100 µl of DMSO was added to dissolve the formed formazan crystals and the plate was further incubated for 5 min at room temperature. Amount of formazan was determined by measuring the optical density at a wavelength of 570 nm using a Micro-plate reader (Biotek: Synergy HT). All experiments were carried out 3 times. The absorbance reading was taken to calculate the percentage of cell survival as follow:

$$\text{(\% cell viability)} = \frac{(OD\ sample)\times 100}{OD\ negative\ control}$$

$$\text{(\% Cytotoxicity)} = \frac{100-(ODsample)\times 100}{OD\ negative\ control}$$

The data were expressed as the concentration of sample required to kill 50% (IC50) of the cells compared to the controls.

Comparison of efficacy of electrolyte water with different disinfectants

The effectiveness of electrolyte water was compared to other disinfectant solutions such as 0.6% v/v sodium hypochlorite, potassium permanganate (0.05%w/v), baking soda mixture (2.5 g/L), and chlorinated water (0.02% w/v). In brief, 100 ul of tested pathogenic microbial suspension (~ 1.5 x 10^5 CFU/ml for bacteria and ~ 1.5 x 10^4 CFU/ml for yeast) was dropped onto the surface of a sterile 5x 5 cm plastic sheet and allowed to dry for about 1 hour. Afterward, 100 ul of various disinfectants were applied to the surface of plastic sheet at difference time point based on the time selected in the previous experiment (3 min, 5 min). The plastic sheet was then immersed in a 1 mL of sterile 0.85% saline solution tube, shaken for 1 min to enable the saline solution to remove bacteria. And after that, 100 µl of the mixture was spread onto the TSA/PDA and incubated at 37°C for 24 hours for bacteria and Candida albicans and at room temperature for 48 hours for Cryptococcus neoformans. Following incubation, the colony that grew on the culture were manually counted and compared to the growth control (sterile NSS) and indicated as a colony forming units (CFUs). The three replicates of individual experiment were performed.

Comparison of the efficacy of electrolyte water by sterilization contact techniques and surface type

The effectiveness of sterilization contact techniques (immersion and spray) and surface type (plastic sheet and fabric clothes) by electrolyte water was evaluated. Briefly, 100 ul of tested pathogenic microbial suspension (~ 1.5 x 10^5 CFU/ml for bacteria and ~ 1.5 x 10^4 CFU/ml for yeast) was dropped onto the surface of difference sterile object (plastic sheet and fabric clothes) and leave to dry for about 1 hour. The following contact techniques were then used as described:

Immersion

The difference object was immersed in 15 mL of electrolyte water at difference time point (3 min, 5 min). After the time has passed, the object was then soaked in a 1 mL of sterile 0.85% saline solution tube, shaken for 1 min to allow the saline solution to remove microbial from the object.

Spray

The surface of difference object was spraying with electrolyte water for 10 sec (15 mL), and then leave the object at difference time point (3 min, 5 min). After the time has passed, the object was then soaked in a 1 mL of sterile 0.85% saline solution tube, shaken for 1 min to allow the saline solution to remove microbial from the object.

Subsequently, 100 µl of mixer were spread onto the TSA/PDA. and incubated at 37°C for 24 hours for bacteria and Candida albicans, and room temp., 48 hours for Cryptococcus neoformans. After incubation, the colony counts on the culture medium was performed by manual counting method compared with the growth control (sterile NSS). The microbial observed after an incubation period, were indicated as a colony forming units (CFUs). The experimental set-up was repeated in triplicate.

Statistical analysis

Each experiment was performed in triplicate and results were expressed as mean ± SD. Data were evaluated by One-way analysis of variance (ANOVA) using SPSS (version 22.0) for significance ($p \leq 0.05$) and the Tukey test at the 95% confidence level.

RESULTS AND DISCUSSION

Electrolyte water chemical test

The electrolyte water had a pH of 8.62±0.02, indicating that its properties were highly alkaline but less as compared to 0.6% Sodium hypochlorite with a very alkaline (pH 12.18±0.05). While distilled water was neutral (pH 7.20±0.05) (Table 1).

Table 1: Chemical properties of sodium hypochlorite, electrolyte water, and distilled water

Tested Substance	Ph	Orp (Mv)	Acc (Ppm)
Electrolyte Water	**8.62±0.02**	114.33±1.53	0.58±0.00
Sodium Hypochlorite	12.18±0.05	309.67±1.15	587.00±0.04
Distilled Water	**.720±.0**05	**125.67±0.58**	**0.00±0.00**

ORP measurement revealed that electrolyte water had an ORP of 114.33±1.53 mV, indicating that it is a medium oxidizing agent, while 0.6% Sodium hypochlorite and distilled water had an ORP of 309.67±1.15 and 125.67±0.58. mV, respectively. Free

chlorine was measuring and found that the electrolyte water had an ACC of 0.58±0.00 ppm, which was lower than the ACC of 0.6% Sodium hypochlorite (587.00±0.04 ppm). Whereas distilled water showed no dissolved free chlorine as shown in Table 1.

Table 2: Chemical stability test, pH and ORP of electrolyte water from day 1 to 7 after production

Day	pH	ORP (mv)
1	8.62±0.02	113.67±1.15
2	8.63±0.01	112.00±1.00
3	8.53±0.02	94.00±1.00
4	8.48±0.01	91.67±1.15
5	8.46±0.02	90.67±0.58
6	8.42±0.01	89.00±1.00
7	8.41±0.01	86.67±1.53
Mean ± SD	8.51± 0.09	96.71± 11.00
%CV	0.01032	0.11369

The stability of electrolyte water was determined. The electrolyte water was stored at room temperature in a sealed container for 7 days, during which time the pH and ORP were continuously measured and compared to the first day of production. It was discovered that the pH of the electrolyte water varied between 8.62±0.02 to 8.41±0.01. The pH decreased slightly after the third day, but there was no significant difference from the first day of production. Whereas the ORP value ranged between +113.67±1.15 to +86.67±1.53, it began to decline on the third day. Table 2 shows that the coefficients of variance (percent CV) were 0.01032 and 0.11369, respectively.

Determination of bactericidal activity by spread plate technique after direct exposure to electrolyte water

The electrolyte water was tested for the inhibiting efficacy properties of clinically important pathogenic microorganisms compared to standard disinfectants. It was found that the quantity of test bacteria (Bacterial Growth control) was in the range of log 2.56±0.13 – log 3.74±0.05 and the tested fungal (Fungal Growth control) was in the range of log 2.46±0.34 - log 2.80±0.03. Electrolyte water is effective in killing both tested bacteria and fungi. It was discovered that the amount number of 8 test pathogens strains was significantly decreased from the first 30 seconds after exposure. All organism was killed within 30 seconds-1 minutes. Whereas the standard disinfectant (0.6% Sodium hypochlorite), was able to kill all tested microorganisms, within 30 seconds-1 minutes too, as shown in Table 3-4.

Table 3: The bactericidal activity test results of 0.6% Sodium hypochlorite (Positive control) were shown in mean±SD from the three identical tests.

Tested organism	Growth control	Viable count (Log CFU/mL)					
		30 sec	1min	3 min	5 min	10 min	15 min
Pseudomonas aeruginosa	3.74±0.05	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*
Acinetobacter baumannii	3.72±0.02	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*
Staphylococcus aureus	3.68±0.05	1.30±0.00	0.00*	0.00*	0.00*	0.00*	0.00*
Bacillus subtilis	2.93±0.05	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*
Listeria Monocytogenes	3.64±0.02	1.19±0.06*	0.00*	0.00*	0.00*	0.00*	0.00*
Edwardsiella tarda	3.01±0.26	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*
Aeromonas hydrophila	2.60±0.03	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*
Salmonella typhi	3.41±0.05	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*
Klebsiella pneumoniae	2.56±0.13	1.50±0.20*	0.00*	0.00*	0.00*	0.00*	0.00*
Shigella flexneri	3.43±0.04	1.40±0.20*	0.00*	0.00*	0.00*	0.00*	0.00*
Candida albicans	2.46±0.34	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*
Cryptococcus neoformans	2.80±0.03	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*

(*) means a statistically significant reduction in the number of bacteria (P<0.05).

Table 4: The bactericidal activity test results of Electrolyte water were shown in mean±SD from the three identical tests.

Tested organism	Growth control	Viable count (Log CFU/mL)					
		30 sec	1min	3 min	5 min	10 min	15 min
Pseudomonas aeruginosa	3.74±0.05	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*
Acinetobacter baumannii	3.72±0.02	1.40±0.17*	0.00*	0.00*	0.00*	0.00*	0.00*
Staphylococcus aureus	3.68±0.05	1.56±0.24*	0.59±0.00	0.00*	0.00*	0.00*	0.00*
Bacillus subtilis	2.93±0.05	1.83±0.21*	1.07±0.75	0.00*	0.00*	0.00*	0.00*
Listeria Monocytogenes	3.64±0.02	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*
Edwardsiella tarda	3.01±0.26	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*
Aeromonas hydrophila	2.60±0.03	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*
Salmonella typhi	3.41±0.05	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*
Klebsiella pneumoniae	2.56±0.13	1.50±0.17*	0.00*	0.00*	0.00*	0.00*	0.00*
Shigella flexneri	3.43±0.04	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*
Candida albicans	2.46±0.34	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*
Cryptococcus neoformans	2.80±0.03	0.00*	0.00*	0.00*	0.00*	0.00*	0.00*

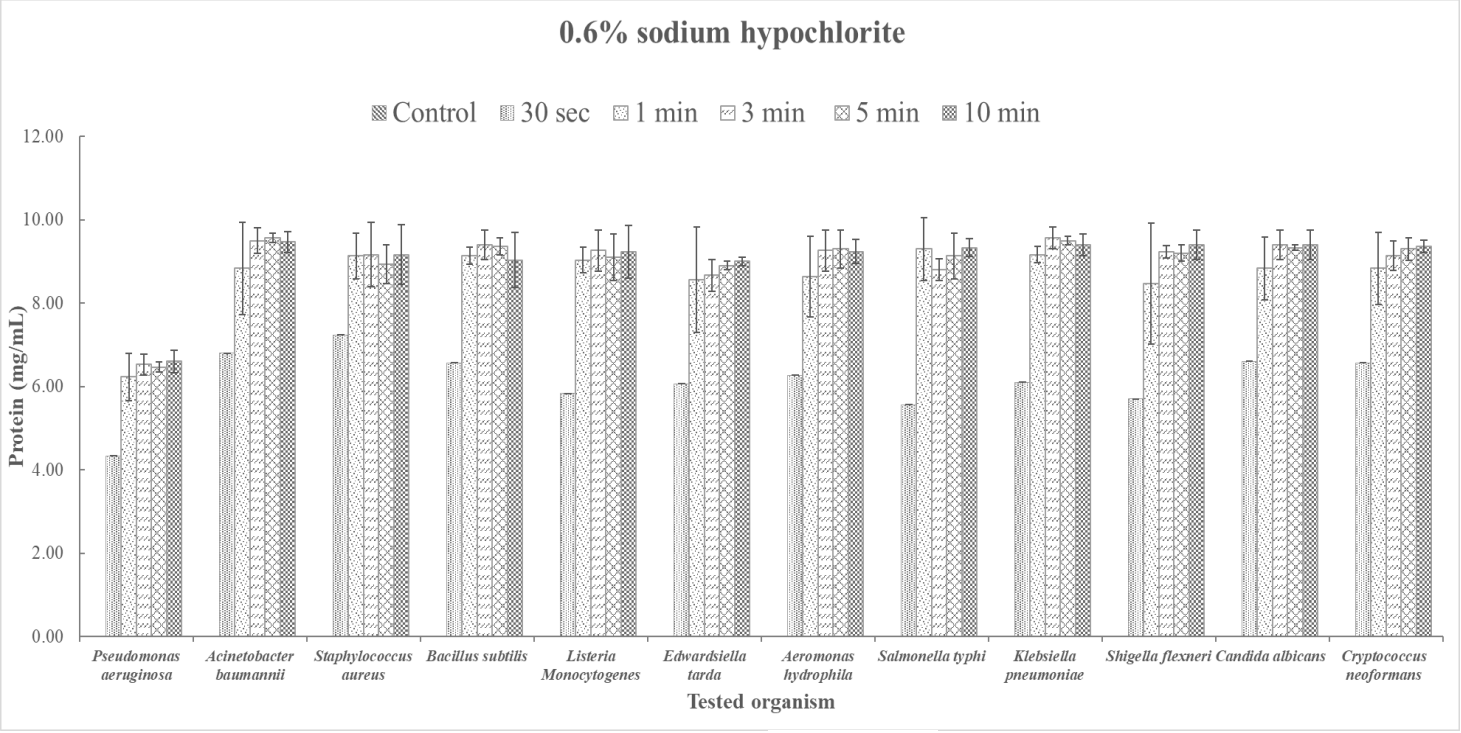
(*) means a statistically significant reduction in the number of bacteria (P<0.05).

Intracellular protein leaking by dye-binding method (Bradford)

Intracellular protein leaking measures the protein that leaks from the breaks down microbial cell. It was found that after the electrolyte water was exposed to the microorganisms at different intervals, proteins from the intracellular organisms of the tested

microorganisms were leaked from 30 seconds and increased at the 1 minute after exposure. Protein concentrations were relatively stable over the following periods at 3 min, 5 min, and 10 min, as shown in figure 1-2.

Figure 1: The amount of protein released from tested microbial cells destroyed with 0.6% Sodium hypochlorite at different times compared with growth control was shown in mean ± SD from the triplicate test.



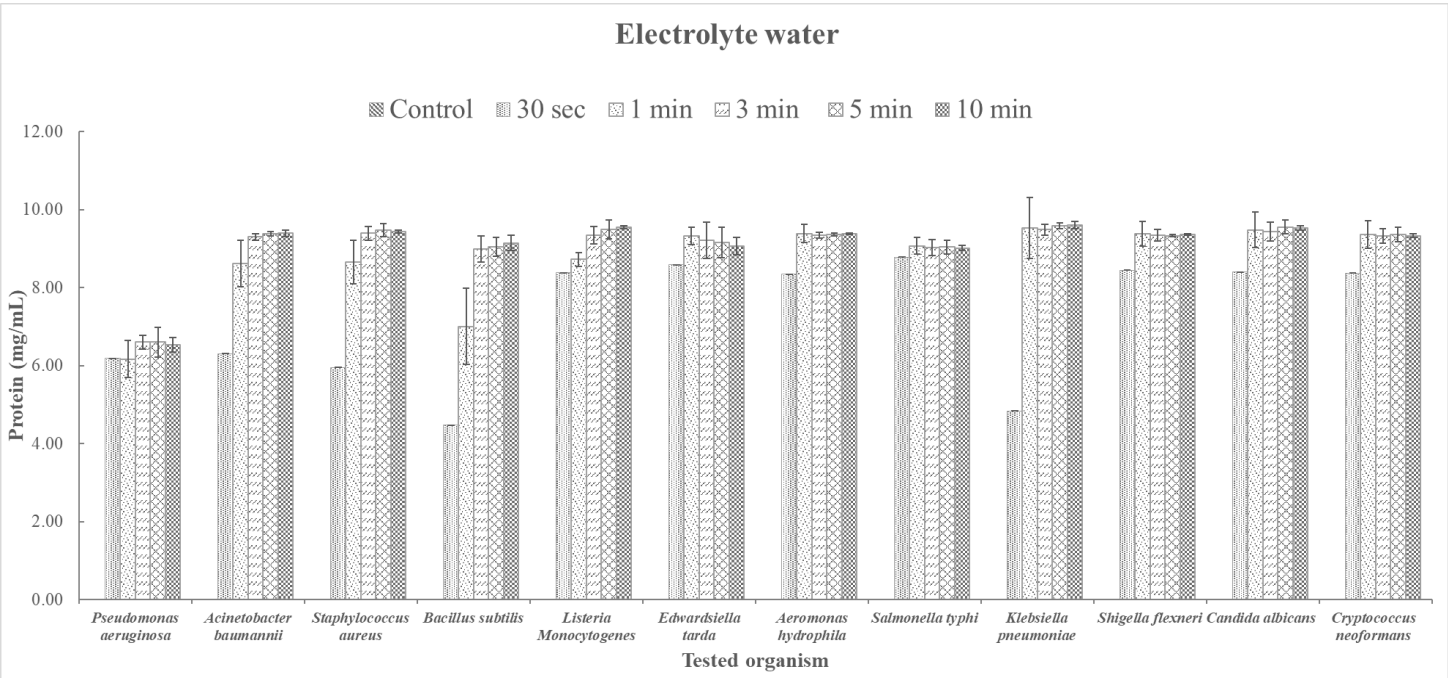
Phenol coefficient test of ozone treated water

Table 5 shows the results obtained when dilutions of ozone-treated water were tested. At 1: 32 and 1: 64 dilutions, growth of B. subtilis and S.Typhi was recorded at 5 minutes, but not at 10 minutes contact times, thus giving a Rideal-Walker Coefficient of 1.00 and 4.00 respectively.

Cytotoxicity of electrolyte water to omuf normal fibroblast

From the cytotoxicity experiment, it was found that at a concentration of 0.1-100 % v/v of electrolyte water can cause a relatively low cytotoxic effect on OMUF fibroblast cells with an IC50±SD value of 80.27 ± 1.26 ug/100 uL, as shown in Figure 3.

Figure 2: The amount of protein released from tested microbial cells destroyed with Electrolyte water at different times compared with growth control was shown in mean ± SD from the triplicate test.



Comparison of efficacy of electrolyte water with different common household disinfectants

It was showed that the amount of test bacteria (Bacterial Growth control) was in the range of log 5.41±0.31– log 6.13±0.02 and the tested fungal (Fungal Growth control) was in the range of log 2.78±0.05 - log 3.05±0.01 respectively. Electrolyte water has antimicrobial properties in all tested microbial. The decreasing in CFU or no growth was detected from 3 minutes after exposure to the electrolyte water. However, the common household disinfectants; 0.2% potassium permanganate, 0.025% chlorine, and 0.5% baking powder, were unable to kill all tested microorganisms at the same time point. As a result, as shown in Table 6, electrolyte water outperformed conventional household disinfectants in terms of disinfectant efficiency.

Comparative results of electrolyte water efficiency in sterilization by various contact techniques

The results from tested plastic sheet showed that the number of tested bacteria (Bacterial Growth control) was in the range of log 5.29±0.13– log 6.02±0.02 and the test fungal (Fungal Growth control) was in the range of log 2.60±0.05- log 3.06±0.04. As a result, from Table 7, it was found that immersion technique can kill most tested pathogen within 3 min after exposed except Salmonella typhi. However, most tested pathogen was killed within 5 min after electrolyte water spraying.

The results from tested cloth pads showed that the number of tested bacteria (Bacterial Growth control) was in the range of log 5.34±0.05– log 6.24±0.05 and the test fungal (Fungal Growth control) was in the range of log 2.47±0.15- log 3.01±0.04. It was found that immersion technique can kill 9 tested pathogens within 5 min after exposed except Bacillus subtilis, Salmonella typhi, and Klebsiella pneumoniae. However, most tested pathogen was killed within 5 min after electrolyte water spraying except Pseudomonas aeruginosa. Whereas the standard disinfectant (0.6% Sodium hypochlorite), was unable to kill most of the tested pathogen from the fabric within 5 minutes, and some microbial growth was still observed as shown in Table 8.

Discussion

Individuals must avoid chemical disinfectants such as benzalkonium chloride, formaldehyde, and glutaraldehyde because they are potentially toxic to humans and corrosive to the application area [8, 9]. The desirable properties of a disinfectant agent are that it is noncytotoxic, destroys microorganisms effectively, and does not damage the living organisms or materials to which it is applied. As a result, investigating the potency of electrolyte water solely in terms of its ability to destroy specific pathogens is insufficient; it is also necessary to assess its toxic effects and develop practical hygiene guidelines.

The results of the electrolyte water chemical test showed that the electrolyte water had a pH of 8.62±0.02, which was alkaline electrolyte water. The oxidation reduction potential (ORP) is moderate and the available chlorine concentration (ACC) is lower than 0.6% sodium hypochlorite. The chemical stability results reveal both pH and ORP of the electrolyte water slightly change from day 3 onwards and stable over 7 days after production indicating that the electrolyte water had good stability.

For anti-bactericidal activity of the electrolyte water, the contact time was tested at 30 sec, 1 min, 3 min, 5 min, 10 min and 15 min to see the minimum time for killing microorganisms. The electrolyte water was found to kill Pseudomonas aeruginosa, Acinetobacter baumannii, Bacillus subtilis, Edwardsiella tarda, Aeromonas hydrophila, Salmonella Typhi, Candida albican and Cryptococcus neoformans from 30 sec after exposure. And it can be disinfected Staphylococcus aureus, Listeria monocytogenes, Shigella Flexner at 1 minute after exposure. This is consistent with research by Yaraksa and coworker [10] reporting that acidic electrolyte water can kill Escherichia coli and Staphylococcus aureus from the first 15 seconds of exposure. The experiment was corresponding to the Intracellular protein leaking results which show that the mechanism for the destruction of microbial cells by electrolyte water is cell rupture and protons leak out of the cell. This was supported by Paola and coworker (2005) [11] who reported that electrolyte water can inhibit the growth of Listeria monocytogenes by damaging the cell walls of microorganisms.

It is essential to rule out the possibility of cytotoxic effects in mammalian cells before using electrolyte water for human disinfection. This parameter was tested and found to be cytotoxic in this mammalian cell line. Electrolyte water cytotoxicity results in a relatively low toxicity effect on OMUF fibroblasts. It was assumed that electrolyte water is safe and harmless to human tissue. Previous research has shown that different concentrations of electrolyte water were not cytotoxic for the L929 mouse fibroblast cell line under 10- to 80-fold dilutions [12].

The efficacy of electrolyte water was comparable to that of phenol, with a Rideal-Walker Coefficient of 1.00 and 4.00 for B. subtilis and S. Typhi, respectively. However, when electrolyte water was compared to various disinfectants commonly used in households, it was found that electrolyte water has ability to kill pathogen better than conventional household disinfectants. Since the amount of tested pathogen was reduced or no growth was detected from 3 minutes after exposure. But the common household disinfectants; 0.2% potassium permanganate, 0.025% chlorine, and 0.5% baking powder, were not able to kill all tested microorganisms at the same time point. These findings in accordance to those reported by Naka and coworker who showing that Electrolyte water has a higher bactericidal activity compared to NaOCl [13].

Comparison of the efficacy of electrolyte water to 0.6% Sodium hypochlorite for disinfection on surfaces of transparent plastic and cloth contaminated with tested pathogen by different contact techniques (immersion and spray). It was found that the electrolyte water was able to sterilize the contamination on the plastic sheet by immersion technique better than spray within 3 minutes after expose. Spraying with electrolyte water, even after 5 minutes, can kill all but Salmonella typhi. While sterilization tested on cloth pads of electrolyte water by spraying is better than soaking because spraying can kill most of the germs in 3 minutes and so on, almost all in 5 minutes. However, even soaking for up to 5 minutes is still unable to completely killed 3 tested pathogens.

However, it was found that 0.6% Sodium hypochlorite was unable to kill most of the tested pathogen from the fabric within 5 minutes, since some microbial growth was still observed.

CONCLUSION

According to the physiochemical and microbiological assay conducted in this study, it was concluded that the electrolyte water used as a disinfectant in this experiment were long self-life, effective in killing all types of pathogen and relatively low toxicity. It has a higher sterilization capacity compared to phenol and conventional household disinfectants such as potassium permanganate, chlorine and baking powder. Disinfecting time using only 1-3 min after exposure. Immersion techniques was the most effective method for cleaning the contaminated object and fabrics which use only 3-5 minutes onwards for disinfecting. Furthermore, electrolyte water was relatively simple to manufacture without the use of any chemicals other than salt, which provides a technological and financial advantage. Therefore, electrolyte water is effective enough to use as a replacement for standard disinfectants.

ACKNOWLEDGEMENTS

The author would like to express her sincere gratitude to the Research Institute of Rangsit University under grant number 52/2563, to P. Powthong for providing additional funding. The author would also like to extend her thanks to Microbiology staff, Faculty of Medical Technology RSU for their providing laboratory equipment and reagent. The authors declare that there is no conflict of interest regarding the publication of this paper.

Conflict of interest statement

The author declares that they have no conflict of interests.

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How to cite this article

Pannapa Powthong, Bajaree Jantrapanukorn, Warangkana Lektrakul, 2022. Antimicrobial activity and safety applications of electrolyzed water as a microbial disinfectant. Journal of medical pharmaceutical and allied science V 11 - I 6, Pages - 5217 – 5223. Doi: 10.55522/jmpas.V11I6.4005.