

The Disinfecting Effect of Electrolyzed Water Produced by BioCharge

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ABSTRACT

BACKGROUND: The use of disinfectants is necessary for hygiene control to prevent food poisoning and for the control against infectious diseases. Evaluation was performed of the disinfecting effect of electrolyzed water produced from BioCharge HOCL generator, electrolysis equipment.

METHODS: Both the strains isolated from the specimens collected from hospitals and the reference strains were exposed to the electrolyzed water produced from BioCharge for 0.5, 1, 2, 5, and 10 min. The mixture was inoculated onto tryptic soy broth/agar, and the bacterial growth and viable count were observed.

RESULTS: General bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus faecium* (VRE), and *E. coli* and trophozoites such as *Bacillus subtilis* were all killed within 1 min after the exposure.

CONCLUSION: The electrolyzed water produced from BioCharge has strong disinfecting effect against general bacteria, and thus can be useful for hygiene control to prevent food poisoning and for control against infectious diseases.

BACKGROUND

Washing, disinfection, sterilization and pasteurization are all a procedure that reduce the number of or remove bacteria, and an important part of approaches for infection control. Washing is a method by which organic matter, soil or microorganisms are removed from certain objects using water, mechanical friction or detergents.

Disinfectant means an agent that can kill infection-causing fungi and trophozoites within 10 min, and does not mean killing of bacterial spores. Disinfectants can be divided into one that can kill bacteria, virus and some fungi but cannot kill tuberculosis bacilli or spores of bacteria (a low grade disinfectant), one that can inactivate tuberculosis bacilli and fungi but cannot kill spores of bacteria (medium grade disinfectant), and one that can even kill spores of bacteria if exposure time is sufficient time (high grade disinfectant). Hypochlorous Acid is classified as a high grade disinfectant by FDA September 18, 2002.

Sanitizer means an agent that can reduce the number of microorganisms that exist on the surface of inorganic substance to the level at which the public health is considered safe. Sterilization means destructing all forms of microorganisms (bacteria, fungi, virus and spores of bacteria) via chemical or physical means.

Sanitizer/disinfectant is an agent that can inhibit the growth of or kills microorganism.

Sanitizer/disinfectant for instruments is a substance that can be used for sterilization and disinfection of instruments, containers or packaging and can be indirectly transferred to food.

This sanitizer/disinfectant can kill 99.999% of trophozoites (5 log₁₀ CFU/mL) within 5 min, and is widely used for food hygiene purposes such as instruments for food, and packaging instruments.

Due to the expansion of catering services, an increase in eating out, westernization of dietary life, global warming and increased indoor temperature, the incidence of food poisoning is on the rise and tends to be large scale involving large number of people.

The most common ingredients used for sterilization/disinfection agents include ammonium, ethanol, chloride, and peroxide families (class IV). Of these, chloride family disinfectants are most commonly used for problems regarding duplicate cost and ease of use.

As infection control becomes active, electrolyzed water, a novel disinfectant agent has been introduced, and its use is expanding. Hypochlorous acid (HOCL) that exerts strong disinfecting effect by electrolyzing water and elemental chloride and electrolyzed oxidizing water that contains active oxygen produced from generator are known to have good disinfecting effect. In this study, assessment was performed of the sterilizing/disinfecting effect of electrolyzed water produced from the BioCharge HOCL generator.

MATERIALS AND METHODS

1. Bacterial Species and Strains.

A total of 9 strains isolated from the specimen collected from patients hospitalized in a college hospital were used: 4 gram negative cocci; methicillin-susceptible *Staphylococcus aureus* (1 strain), methicillin-resistant *Staphylococcus aureus* (1 strain), vancomycin-resistant *Enterococcus faecium* (1 strain), *Enterococcus faecalis* (1 strain) and 9 gram negative bacilli; *Escherichia coli* (1 strain), *Salmonella Typhi* (1 strain), *Salmonella Enteritidis* (1 strain), *Shigella sonnei* (1 strain), *Pseudomonas aeruginosa* (1 strain), *Acinetobacter baumannii* (1 strain), *Klebsiella pneumoniae* (1 strain), *Enterobacter cloacae* (1 strain), *Stenotrophomonas maltophilia* (1 strain). Reference strains included *Staphylococcus aureus* ATCC 29213, *E. coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Bacillus subtilis* ATCC 6633.

2. Preparation of Sterilizing/disinfecting Water (Electrolyzed Water)

Sterilizing/disinfecting water was produced using BioCharge HOCL generator, a electrolyzed water-producing equipment according to the instruction from the manufacturer: tap water 1.2 L and salt 2 g were put in the electrolyzing reactor and electrolysis was performed for 3 min. According to the instruction from the manufacturer of BioCharge, the chloride concentration of the electrolyzed water, if produced following the instruction, would be 20-90 ppm.

3. Assessment of Disinfecting Effect

Pure cultured strains were cultured in tryptic soy broth (TSB) for 18 hrs. The cultured colony was put into physiological saline, centrifuged at 3,000 rpm for 15 min, and then the supernatants were removed. Phosphate buffer 10 ml (pH 7.2) was added to the precipitated bacteria, suspended using mixer, and the turbidity was set at McFarland 3.

Each (0.5 ml) of the bacterial liquids was put in the physiological saline 4.5 ml, serial dilution was performed from 10 to 10⁷ folds in TSB to achieve final bacterial count 10⁷-10⁸ CFU/mL. Each (0.5 ml) of the bacterial liquids was added to the electrolyzed water 4.5 ml, and left in the room temperature; 0.1 ml each was inoculated onto the TSB broth 5 ml with exposure time set at 0.5, 1, 2, 5 and 10 min and cultured in tryptic soy agar (TSA) using platinum loop 0.01 mL. Then the growth and viable count was calculated.

The criteria of disinfecting effect of the sterilizing/disinfecting agent was defined as viable count being decreased to 5 log 10 folds or less in 5 min after the treatment with sterilizing/disinfecting agent.

RESULTS

1. Disinfecting Effect Against Gram Negative Cocci

For all strains (methicillin-susceptible *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant *Enterococcus faecium*, *Enterococcus faecalis*, and

Staphylococcus aureus ATCC 29213), the viable count decreased to 5 log₁₀ within 30 sec after the exposure (Table 1).

2. Disinfecting Effect Against Gram Negative Bacilli

For all strains (*E. coli*, *Salmonella Typhi*, *Salmonella Enteritidis*, *Shigella sonnei*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Stenotrophomonas maltophilia*, *E. coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853) the viable count decreased to 5 log₁₀ within 1 min after the exposure (Table 1).

3. Disinfecting Effect Against *Bacillus subtilis*

Viable count of *Bacillus subtilis* ATCC 6633, a trophozoite decreased to 5 log₁₀ within 1 min after the exposure (Table 1).

Table 1. Biocidal activity of electrolyzed water produced by BioCharge HOCL generator against various microorganisms according to exposure time

Test microorganism	Initial Count (CFU/ml)	Colonies remaining (CFU/ml)				
		0.5min	1min	2min	5min	10min
Methicillin-susceptible <i>Staphylococcus aureus</i>	1.5x10 ⁸	<10 ²	<10 ²	<10 ²	<10 ²	<10 ²
Methicillin-resistant <i>Staphylococcus aureus</i>	1.3x10 ⁸	<10 ²	<10 ²	<10 ²	<10 ²	<10 ²
Vancomycin-resistant <i>Enterococcus faecium</i>	1.1x10 ⁸	<10 ²	<10 ²	<10 ²	<10 ²	<10 ²
<i>Enterococcus faecalis</i>	1.2x10 ⁸	<10 ²	<10 ²	<10 ²	<10 ²	<10 ²
<i>Escherichia coli</i>	1.6x10 ⁸	<10 ²	<10 ²	<10 ²	<10 ²	<10 ²
<i>Klebsiella pneumonia</i>	1.3x10 ⁸	<10 ²	<10 ²	<10 ²	<10 ²	<10 ²
<i>Enterobacter cloacae</i>	1.2x10 ⁸	<10 ²	<10 ²	<10 ²	<10 ²	<10 ²
<i>Salmonella Typhi</i>	1.4x10 ⁸	<10 ²	<10 ²	<10 ²	<10 ²	<10 ²
<i>Salmonella Enteritidis</i>	1.8x10 ⁸	<10 ²	<10 ²	<10 ²	<10 ²	<10 ²
<i>Shigella sonnei</i>	1.0x10 ⁸	<10 ²	<10 ²	<10 ²	<10 ²	<10 ²
<i>Pseudomonas aeruginosa</i>	1.2x10 ⁸	<10 ²	<10 ²	<10 ²	<10 ²	<10 ²
<i>Acinetobacter baumannii</i>	1.4x10 ⁸	<10 ²	<10 ²	<10 ²	<10 ²	<10 ²
<i>Stenotrophomonas maltophilia</i>	1.5x10 ⁸	<10 ²	<10 ²	<10 ²	<10 ²	<10 ²
<i>Staphylococcus aureus</i> , ATCC 29213	1.2x10 ⁸	<10 ²	<10 ²	<10 ²	<10 ²	<10 ²
<i>E. Coli</i> . ATCC 25922	1.3x10 ⁸	<10 ²	<10 ²	<10 ²	<10 ²	<10 ²
<i>Pseudomonas aeruginosa</i> , ATCC 27853	1.5x10 ⁸	<10 ²	<10 ²	<10 ²	<10 ²	<10 ²
<i>Bacillus subtilis</i> , ATCC 6633 (vegetative form)	5.3x10 ⁷	<10 ²	<10 ²	<10 ²	<10 ²	<10 ²

CONCLUSION

The electrolyzed water produced from the BioCharge HOCL generator electrolyzer has strong disinfecting effect against general bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus faecium* (VRE), *E. coli*, *Salmonella*, *Shigella*, and thus it is believed that it can be useful as a sterilizing/disinfecting agent for hygiene control to prevent the occurrence of food poisoning and for control against infectious diseases.