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Sterilization of Spent Bathwater and Washed Fabrics by the Addition of Weakly Acidic Electrolyzed Water

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Abstract: This paper attempts to propose a laundry use of spent bathwater in Japan sterilized by non-diaphragm electrolytic treatment (Single cell). Twenty liters of spent bathwater was treated with 25 mL of electrolyzed water obtained by electrolytic treatment to examine its bactericidal effect. The treated bathwater also was used for washing fabrics to examine the antibacterial effect of the treatment on the washed fabrics. Before the experiment, non-treated bathwater was used for washing fabrics and revealed that the washed fabrics had as many as 8.19×10^3 CFU/mL viable bacteria. When distilled water was used for "rinsing" the washed fabrics, many viable bacteria remained on the washed fabrics, even after "rinsing" for 12 minutes. Addition of 25 mL of aqueous HCl to 20 L of spent bathwater indicated that the acid did not have any bactericidal effect. In contrast, addition of 25 mL of electrolyzed aqueous HCl to 20 L of spent bathwater almost completely eliminated viable bacteria. Moreover, when the treated bathwater was used for washing fabrics dipped in the spent bathwater, few viable bacteria were found on the washed fabrics. These results demonstrate that non-diaphragm electrolytic treatment was very effective at killing bacteria in spent bathwater and at sterilizing fabrics washed in the treated bathwater.

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1. Introduction

Among the water used in Japanese homes, water for bathing accounts for 32%, followed by water for laundry at 22%. Thus, these two purposes account for more than half of domestic water use [1]. Therefore, the most feasible and efficient method for saving water domestically is to find a use for spent bathwater. The use of spent bathwater for laundry could greatly contribute to water saving. In addition, since spent bathwater has a higher temperature than tap water, it is well-suited for the removal of attached dirt.

However, when spent bathwater is used for laundry, odors due to bacterial growth often remain on the clothing. Since the optimal temperature for bacterial propagation is 30-37°C, spent bathwater is a suitable medium for bacterial growth. Spent bathwater contains large numbers of bacteria, and may contain harmful bacteria such as *Legionella* bacteria. *Legionella* bacteria can survive at temperatures up to 60°C, and cases of infection at public baths have been reported [2,3].

To overcome these challenges, ways to utilize spent bathwater in laundry in a safe and hygienic way were investigated, leading to treatment of spent bathwater addition of electrolytic treated water from a non-diaphragm process (Single cell). The results indicate that electrolyzed water has a sterilizing effect on spent bathwater, and can sterilize fabrics washed in the treated bathwater.

2. Experimental

2.1 Sampling method for spent bathwater

Samples of spent bathwater was collected at 7 : 00 AM on a day in mid-April from a household composed of a husband, a wife, and a junior high school child.

2.2 Preparation of electrolyzed water

A schematic representation of the non-diaphragm electrolytic process (Hokuty Co., Ltd., Piamini) used in this experiment is shown in Fig. 1. Different concentrations (0.5, 1.0, 2.0, and 3.0 N) of aqueous HCl were injected (25 mL) into an electrolysis cell, and electrolytic treatment was performed for a predetermined period. The electrolytic voltage was set at 5.0 V. At the end of the electrolytic treatment, a shutter placed at the bottom of the electrolysis cell was opened, and 25 mL of generated electrolyzed water was added to 20 L of spent bathwater. The electrolysis cell was installed in the tank

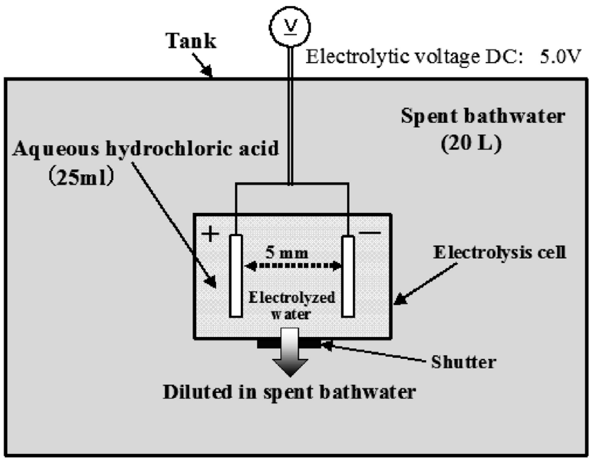

Fig. 1 Schematic representation of non-diaphragm electrolytic process (Single cell).

containing the spent bathwater to prevent a temperature rise inside the electrolysis cell and to prevent the chlorine odor from entering the room.

2.3 Laundry method

Each type of water (100 mL each) was placed into a washing bottle (430 mL) together with 10 steel balls (6.3 mm φ), and the bottle was immersed in warm water (40°C) for 10 min to maintain a constant temperature inside the bottle. Then, two pieces of cotton fabric (Shikisensha Co., Ltd., cotton broad cloth), 5 cm × 5 cm, were added, and "washing" was performed for 30 min at 40°C and 42 rpm using a KL-8 launder-ometer made by Koa-shokai Co., Ltd. "Rinsing" was achieved using a batch method and the washed fabrics were air dried after rinsing.

2.4 Measurement

2.4.1 Optical Microscopy

Bacterial colonies were collected from test tubes using a Pasteur pipette or from Petri dishes using a needle, followed by examination under a light microscope (Olympus BX60) for identification. An Olympus DP-12 camera was used for photography.

2.4.2 Number of viable bacteria in washing water

An aqueous solution of 0.5 wt% yeast extract (Becton Dickinson), 1.0 wt% pepton (Becton Dickinson), 0.5 wt% sodium chloride, and 1.5 wt% agar was sterilized in an autoclave at 121°C for 15 min. This aqueous solution (*ca*. 20 mL) was transferred to a Petri dish and was allowed to sit for 30 min while a standard agar medium for general bacteria was prepared. In addition, a 5.0 wt% aqueous MacConkey agar medium (Nissui Pharmaceutical Co., Ltd.) was sterilized in an autoclave at 121°C for 15 min. This aqueous solution (*ca*. 20 mL) was transferred to a Petri dish and was allowed to sit for 30 min while the medium for *Escherichia coli* was prepared. To the prepared standard agar medium and *Escherichia coli* medium plates were added 0.1 mL and 1.0 mL of the test solutions, followed by inverted incubation at 37°C for 46 h. The number of bacterial colonies was counted visually and the value converted to the number of viable bacteria per 1.0 mL of the sample solution. This measurement was performed 6 times, and average value was shown. Culture media with 1.0-mL additions of the test solutions were photographed.

2.4.3 Number of viable bacteria attached to fabrics for laundry

Wet fabric after laundry was placed into a vial and kept warm at 37°C for 20 h. After heating, a "washing-out solution" with a volume four times that of the liquid contained in the fabric was added to the vial and the mixture was agitated. Then, 0.1 mL and 1.0 mL portions of the extract washed from the fabric by "the washing-out solution" were dropped onto standard agar media, and the media were subjected to inverted incubation at 37°C for 46 h. The number of bacterial colonies was counted visually and the value converted to the number of viable bacteria per 1.0 mL of sample solution. This measurement was performed 6 times, and average value was shown. "The washing-out solution" was prepared by sterilizing an aqueous solution composed of 0.85 wt% sodium chloride and 0.20 wt% Tween 80 (Sigma Chemical Co., Ltd.) in an autoclave at 121°C for 15 min. The media with the addition of 1.0 mL of the test solution were photographed.

2.4.4 Free residual chlorine concentration

To measure the amount of free residual chlorine, a DPD (N,N-diethyl-*p*-phenylenediamine) method using an instrument made by Sibata Scientific Technology Ltd. was used. DPD reagent (0.12 g) was added to a 10 mL test solution, and the color change at 5 sec was correlated with standard colorimetric plates to obtain the free residual chlorine concentration [4].

3. Results and discussion

3.1 Removal of viable bacteria attached to washed fabrics by rinsing

Testing was done to determine if the viable bacteria that remain attached to fabrics washed in spent bathwater could be washed out by "rinsing." After "washing" cotton fabrics in spent bathwater without adding detergent, distilled water was used for "rinsing." The rinsing time was 3 min per cycle and several cycles were repeated, and one prolonged period of "rinsing" was applied. By changing the pattern of rinsing, the total rinsing time

Table 1 Number of viable bacteria number on fabrics after washing in spent bathwater wastes and rinsing with distilled water under different conditions.

	Condition of rinsing (Distilled water)			Viable bacteria (CFU/ml)
	Time (min.)	Cycle (—)	Total time (min.)	
	no rinsing		0	3.18×10^4
Changes of rinse cycle	3	**1**	3	1.88×10^4
	3	**2**	6	1.57×10^4
	3	**3**	9	1.36×10^4
	3	**4**	12	1.19×10^4
Changes of rinsing time	**3**	1	3	1.88×10^4
	6	1	6	1.64×10^4
	12	1	12	9.70×10^3

could be adjusted from 3 to 12 min. In this experiment, rinsing was conducted using a batch method while replacing the distilled water with fresh water each time.

The number of viable bacteria attached to fabrics for laundry after rinsing was measured. The results are shown in Table 1. Even when a 3-min rinse was repeated four times, or a 12-min rinse was performed, about the same number of viable bacteria were present on the rinsed fabric as on the unrinsed fabric (*ca*. 10^4 CFU/mL). Once "washing" was performed using the spent bathwater, bacteria attached to fabrics were difficult to remove, even through rinsing was conducted for a long period using distilled water. In addition, when "washing" is performed using spent bathwater, fabrics retain an unpleasant odor, even after repeated rinsing in distilled water.

3.2 Difference in sterilizing effects of HOCl and NaClO

To use spent bathwater for laundry safely, it is necessary to sterilize it. Sterilization with hypochlorous acid (HOCl) has been done [5,6]. However, in the pH range of 3.0 or below, a high level of harmful chlorine (Cl_2) gas is likely to be produced [7]. In contrast, in water with a pH between 3.0 and 5.5, the generation of chlorine gas is minimal, and a large amount of hypochlorous acid is present [7]. When the pH of the water is greater than 5.5, the amount of hypochlorous acid decreases again, and the ratio of hypochlorite ions (OCl^-) increases [7].

According to the sterilizing power test approved by the United States Environmental Protection Agency (EPA) using an *Escherichia coli* complex, hypochlorous acid is about 80-fold better than hypochlorite ions at killing 99% of bacteria in water at temperatures between 2 and 6°C [7]. Thus, hypochlorous acid has greater sterilizing power compared to hypochlorite ions.

Aqueous sodium hypochlorite solution, a common disinfectant, is generally used at a concentration between 100 and 200 ppm. The pH in this concentration range is approximately 9, which indicates a low level of hypochlorous acid, which is the sterilizing agent. To achieve conditions that effectively generate hypochlorous acid, it is necessary to control the pH between 3.0 and 5.5.

An experiment was conducted to adjust the pH by adding acid to aqueous sodium hypochlorite. Titration was performed by adding 0.1 M HCl to 100 mL of 0.1 M NaOCl, while measuring the change in pH (Fig. 2). The results showed a steep change in pH between 3.0 and 5.5 against the HCl titrant volume. Thus, this method is technically unfeasible for controlling the pH between 3.0 and 5.5 when the hypochlorous acid concentration is large.

3.3 Property changes due to electrolysis of HCl solution

To develop an effective method for generating hypochlorous acid, the electrolysis of aqueous HCl was examined [8]. In a non-diaphragm electrolytic process, a chemical reaction such as $2Cl^- \rightarrow Cl_2 + 2e^-$ occurs at the anode, while the reaction $2H^+ + 2e^- \rightarrow H_2$ occurs at the cathode. For compounds generated by electrolysis, Cl_2 is water-soluble and H_2 is water-insoluble. As a result, the chemical change in water is expressed as $Cl_2 + 2H_2O \rightarrow 2HClO + H_2\uparrow$. When these reactions are summarized, HClO and H_2 are generated through electrolysis. Thus, hypochlorous acid can be generated relatively and easily using a non-diaphragm electrolytic treatment [6].

In this experiment, a non-diaphragm electrolytic treatment was applied to different concentrations of aqueous HCl (25 mL), and the electrolyzed water generated was added into 20 L of spent bathwater. Table 2 compares the physicochemical properties of the spent bathwater when HCl was added to it and when HCl after electrolytic treatment was added to it. Treated water

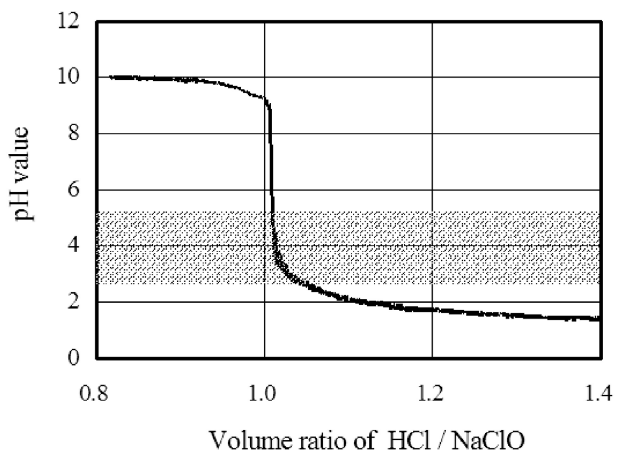

Fig. 2 pH titration curve of hydrochloric acid / sodium hypochlorite.

Table 2 Physicochemical properties of spent bathwater wastes treated with electrolyzed water under different electrolytic treatment conditions.

Sample (Spent bathwater with electrolyzed water)	Condition of electrolytic treatment		Spent bathwater **with non-electrolyzed water**		Spent bathwater **with electrolyzed water**	
	Concentration of electrolyte (HCl solution)	Time of Electrolytic Treatment (min.)	pH	Free residual chlorine concentration (mg/l)	pH	Free residual chlorine concentration (mg/l)
Untreated spent bathwater	—	—	7.05	0	—	—
Treated water -1	**0.5 N**	10	5.20	0	5.90	2
Treated water -2	**1.0 N**	10	3.17	0	4.79	6
Treated water -3	**2.0 N**	10	2.82	0	4.17	20
Treated water -4	**3.0 N**	**10**	2.62	0	3.97	23
Treated water -5	3.0 N	**20**	2.62	0	3.56	60
Treated water -6	3.0 N	**30**	2.62	0	3.37	90

samples 1 - 4 involved different concentrations of HCl solution that were treated with electrolysis for 10 min and subsequently added to 20 L of spent bathwater. Treated water samples 4 - 6 involved a 3.0 N HCl solution treated with electrolysis for varying lengths of time and subsequently added to 20 L of spent bathwater.

The results showed that addition of a HCl solution without electrolytic treatment to spent bathwater resulted in a concentration of 0 mg/L free residual chlorine. Thus, no free residual chlorine was generated when a HCl solution with no electrolytic treatment was added to spent bathwater. In contrast, addition of a HCl solution after electrolytic treatment resulted in a concentration of 2-90 mg/L free residual chlorine in the spent bathwater. The greater the HCl concentration of the solution added to the electrolysis cell, the greater the concentration of free residual chlorine in the bathwater. For example, when the HCl solution in the electrolysis cell was 0.5 N, the free residual chlorine concentration in the spent bathwater treated with electrolyzed water was 2 mg/L. In contrast, when a 3.0 N HCl solution was treated with electrolysis and added to spent bathwater, the free residual chlorine concentration reached 23 mg/L. Even when the HCl solution was 3.0 N, if the electrolytic treatment time was extended to 30 min, the free residual chlorine concentration in the treated bathwater increased to 90 mg/L. In the treated water containing a high concentration of free residual chlorine, the concentration of hypochlorous acid should also be high. Thus, the greater the free residual chlorine concentration, the greater the level of hypochlorous acid for sterilization.

The greater the HCl solution concentration, the lower the pH value was of the spent bathwater treated with the HCl solution. For all samples, the pH of the spent bathwater treated with an electrolyzed HCl solution tended to be slightly higher than that of bathwater treated with a non-electrolyzed HCl solution. As the electrolytic treatment time increased, the pH of bathwater treated with electrolyzed water decreased (Table 2).

3.4 Sterilization using electrolyzed aqueous HCl solution

The ability of an electrolyzed aqueous HCl solution to sterilize spent bathwater was next examined. Figure 3 shows optical microscopic images of spent bathwater treated with electrolyzed water. The photograph in the upper left is an optical microscopic image of the bathwater before treatment as a reference. The results demonstrate that live bacteria (2-3 μm in size) were present in the untreated bathwater but none were found in the treated bathwater.

Table 3 shows the number of live bacteria in spent bathwater treated with an electrolytic HCl solution and in bathwater treated with a non-electrolytic HCl solution. Figure 4 shows photographs of the propagation of bacteria in a standard agar medium from spent bathwater treated with electrolyzed water. The untreated bathwater contained live bacteria at levels of approximately 10^3 CFU/mL (Table 3). In addition, viable bacteria were found in bathwater treated with an aqueous HCl solution at all concentrations (Table 3). Thus, bacteria in the spent bathwater did not perish when a HCl solution alone was added, indicating that HCl had no sterilizing effect at the concentrations tested. Sterilization of the bathwater occurred only upon addition of an electrolytically treated HCl solution.

Electrolyzed HCl solutions produced a sterilizing

Table 3 Number of viable bacteria in spent bathwater wastes treated with electrolyzed water at different electrolyte concentrations (aqueous HCl solution).

（Unit: CFU/ml）

	Item	Untreated spent bathwater	Spent bathwater with electrolyzed water*1)					Distilled water
			Concentration of HCl aqueous solution as electrolytes					
			—	0.5 N	1.0 N	2.0 N	3.0 N	
Spent bathwater with **non-electrolyzed water**	pH	7.05	7.05	5.20	3.17	2.82	2.62	5.50
	General bacteria	**9.15×10^3**	**9.15×10^3**	**8.97×10^3**	**7.16×10^3**	**7.43×10^3**	**7.04×10^3**	—
	Escherichia coli	**5.0×10**	**5.0×10**	**8**	**1**	**0**	**0**	**0**
Spent bathwater with **electrolyzed water**	pH	—	6.92	5.35	4.08	3.51	3.48	—
	General bacteria	—	**4.25×10^3**	**0**	**10**	**10**	**0**	—
	Escherichia coli	—	**3.0×10**	**0**	**0**	**0**	**0**	—

＊1）Spent bathwater: 20L, HCl aqueous solution: 25ml

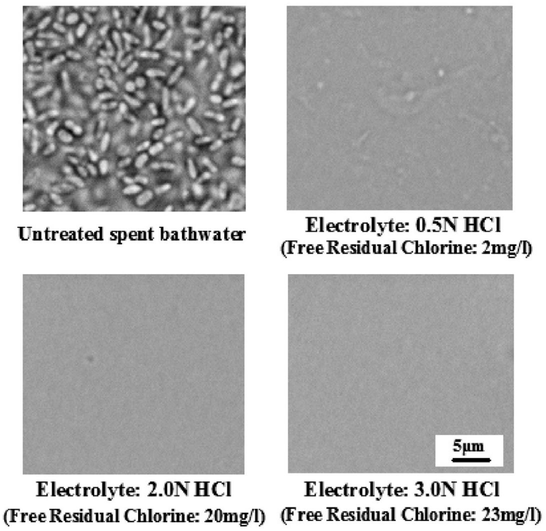

Fig. 3 Optical microscopic images of bacteria in spent bathwater wastes treated with electrolyzed water at different electrolyte concentrations (aqueous HCl solution) (×1,000).

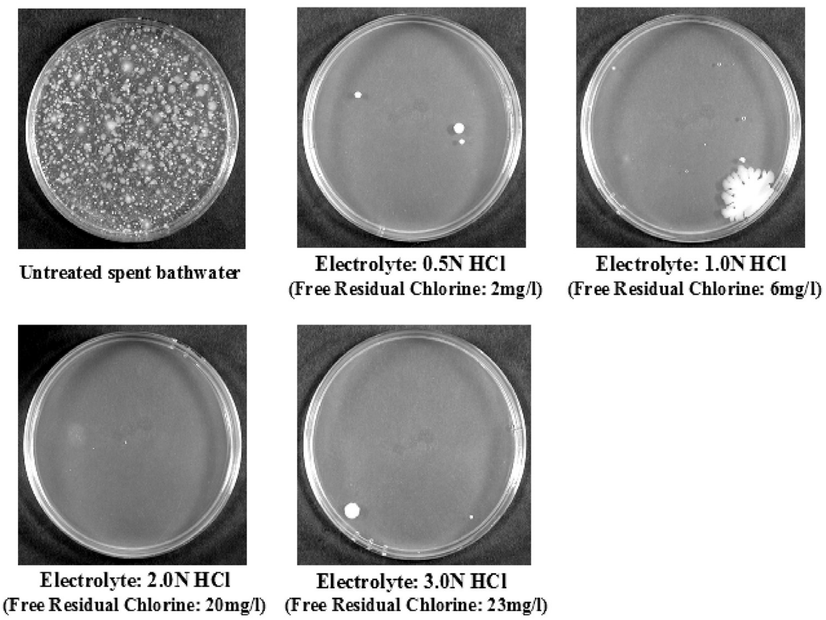

Fig. 4 Bacteria remaining in spent bathwater wastes treated with electrolyzed water at different electrolyte concentrations (aqueous HCl solution).

Table 4 Number of viable bacteria from fabrics washed in spent bathwater wastes treated with electrolyzed water at different electrolyte concentrations (aqueous HCl solution).

（Unit: CFU/ml）

Untreated spent bathwater	Spent bathwater with electrolyzed water*1) Concentration of HCl aqueous solution as electrolytes 0.5 N	1.0 N	2.0 N	3.0 N	Distilled water
8.19×10^3	5	10	15	5	5

＊1）Spent bathwater: 20 L, HCl aqueous solution: 25ml

effect on bacteria in spent bathwater. When electrolyzed aqueous HCl solutions at concentrations of 0.5 N or above were added to spent bathwater, viable bacteria in the bathwater decreased to about 10 CFU/mL (Table 3). In contrast, incubation using a standard agar medium revealed that few bacterial colonies grew from bathwater treated with electrolyzed water. This clearly demonstrates the sterilizing power of electrolyzed water (Fig. 4).

Viable *Escherichia coli* were found in spent bathwater after addition of 25 mL of 0.5 N HCl to 20 L of the bathwater. However, addition of an aqueous HCl solution at concentrations of 1.0 N or greater killed most of the bacteria (Table 3). Thus, addition of an aqueous HCl solution alone had a sterilizing effect on *Escherichia coli*.

3.5 Ability of electrolyzed water to sterilize washed fabrics

Addition of an aqueous HCl solution after electrolytic treatment to spent bathwater had a sterilizing effect. However, to determine whether electrolyzed water could sterilize fabric washed in the bathwater, bathwater treated with electrolyzed water was used to “wash” fabrics for 30 min. To avoid any sterilizing effect due to detergent or rinsing, no detergent was used and no rinsing was done.

Table 4 shows the number of viable bacteria attached to fabrics after washing. The results show that up to 8.19×10^3 CFU/mL bacteria remained attached to the fabric washed in untreated bathwater (Table 4). In contrast, when spent bathwater treated with electrolyzesolutions at concentrations greater than 0.5 N was used for washing, only 10 CFU/mL of viable bacteria were attached to the fabric (Table 4). Thus, bathwater treated with electrolyzed water is also effective for the sterilization of washed fabrics.

3.6 Sterilizing effect of sodium hypochlorite on spent bathwater

Sodium hypochlorite, a chlorine bleach, is generally used to sterilize fabrics. Predetermined concentrations of sodium hypochlorite were added to spent bathwater to examine its sterilizing effect [9]. Table 5 shows the pH and free residual chlorine concentrations of spent bathwater after treatment with sodium hypochlorite. The results indicate that, although the addition of sodium hypochlorite caused a slight increase in pH, the value remained nearly neutral, ranging between 6.8 and 7.2. In contrast, the free residual chlorine concentration was 0 mg/L upon addition of sodium hypochlorite at 3.0×10^{-3} wt% or less, and was still very low at 0.1 mg/L even upon addition of 6.0×10^{-3} wt%. When the amount of sodium hypochlorite was increased to 1.0×10^{-1} wt%, the free residual chlorine concentration increased to 24 mg/L.

Table 5 shows the number of viable bacteria upon addition of sodium hypochlorite to spent bathwater. The results showed that sodium hypochlorite at levels of 6.0×10^{-3} wt% or below resulted in 10^3 CFU/mL viable bacteria remaining, which was the same value found for untreated bathwater. In contrast, levels of 1.0×10^{-2} wt% or above killed nearly all the bacteria in the spent bathwater. Thus, sterilization of spent bathwater required addition of sodium hypochlorite at levels of 1.0×10^{-2}% or greater, to produce a concentration of free residual chlorine of at least 0.2 mg/L. Thus, this method requires addition of a

Table 5 Number of viable bacteria in spent bathwater wastes treated with different concentrations of sodium hypochlorite.

Concentration of NaClO（wt%）	pH	Free residual chlorine concentration (mg/l)	Viable bacteria（CFU/ml）
Non-additive	6.96	0	9.79×10^3
1.0×10^{-4}	6.79	0	9.20×10^3
1.0×10^{-3}	6.90	0	9.56×10^3
3.0×10^{-3}	7.05	0	6.42×10^3
6.0×10^{-3}	7.06	0.1	1.60×10^3
1.0×10^{-2}	6.90	0.2	3
1.0×10^{-1}	7.21	24	0

large amount of sodium hypochlorite, and the strong sterilizing ability of hypochlorous acid is not due to a high pH value. For this reason, a large amount of sodium hypochlorite is required to obtain sufficient sterilization power.

Also, addition of sodium hypochlorite to washing water leaves a sodium hypochlorite residue on washed fabrics if not rinsed extremely well, and drainage water containing sodium hypochlorite can be harmful to the environment. Chlorine gas can also be generated if a handling error is made, which means strict control procedures must be in place. When considering the sterilization of foods, a non-diaphragm electrolytic treatment process has the advantage of leaving no residue.

When the spent bathwater after electrolytic treatment was utilized for washing, the effects of hypochrolous acid on fiber material, detergent, and dyed material are considered. In fact, slight damage after washing is observed in some fiber materials. In addition, a phenomenon such as slight decrease in detergent efficiency is observed. As a countermeasure for this, examination of the use of electrolyzed water for only "rinsing" without the use for "washing" was conducted. In addition, the effect of clear decrease in odor due to the addition of electrolyzed water to spent bathwater was discovered. These results will be summarized and reported in another paper [10]. Future research will examine the application of the non-diaphragm electrolytic process to treat items other than fabrics.

4. Conclusions

Electrolyzed water generated through a non-diaphragm electrolytic process was investigated to determine its ability to sterilize spent bathwater and to determine the ability of the treated bathwater to launder fabrics.

(1) When spent bathwater was used to "wash" fabrics for 30 min, viable bacteria (8.19×10^3 CFU) remained on the fabrics. A long period of rinsing with distilled water could not remove these bacteria, leaving a number of viable bacteria on the washed fabrics after rinsing.

(2) When aqueous HCl was added to spent bathwater, 10^4 CFU of bacteria remained, indicating the inability of the HCl to sterilize the bathwater. In contrast, an electrolytically treated HCl solution eliminated almost all viable bacteria after addition to spent bathwater.

(3) After treatment of spent bathwater with an electrolytic HCl solution, fabrics washed in the treated bathwater contained few bacteria (10 CFU/mL), indicating that electrolyzed water from a non-diaphragm process is not only effective for the sterilization of spent bathwater but also for the sterilization of fabrics washed in the treated bathwater. Therefore, the electrolytic treatment process can lead to recycling of the wasted water for laundry usesage.

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References

1. H. Kaji, *Better Living*, 155, 49-52 (1997).
2. A. Ishikawa, J. Okada, H. Kondo, Y. Takayama, K. Sunagawa, T. Enari and Y. Ishii, *J. Jpn. Asso. Infectious Diseases*, **78**, 898-904 (2004).
3. M. Irie, S. Yoshida, H. Miyamoto and M. Ikeda, *J. Occup. Health*, **46**, 68-77 (2004).
4. J. N. Jensen and J. D. Johnson, *J. Am. Water Works Assoc*., **81**, 59-64 (1989).
5. A. Iwasawa, Y. Nakamura, A. Harano, Y. Akiyama and Y. Nishimoto, *Bokin Bobai*, **37**, 243-252 (2009).
6. M. Okamoto, Y. Komagata, K. Komiyama, S. Okuda, Y. Nishimoto, M. Kamoshida and T. Nakamura, *Bokin Bobai*, **34**, 3-10 (2006).
7. N. Tanbo and K. Ogasawara, "*Jyosui no Gijyutsu*", 101-106, Gihodo Shuppan (1999).
8. S. Yi-Cheng, L. Chengchu and H. Yen-Con, *ACS Symp. Ser. (Am. Chem. Soc.)*, **967**, 309-322 (2007).
9. W. Roeske and C. Mueller, *Brauwelt*, **143**, 287-288, 290-292 (2003).
10. T. Takahashi, Y. Tsurunaga, Y. Aso, and T. Kondo, *Sen'i Gakkaishi*, **68**, 149-155 (2012).