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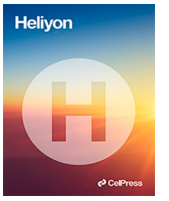

Research article

Kinetics of sterilization of atomized slightly acidic electrolyzed water on tableware

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ABSTRACT

The aim of this study was to elucidate the kinetics of atomization of slightly acidic electrolyzed water (SAEW) for use in sterilization of secondary contaminated tableware surfaces. The sterilization efficacy of SAEW was assessed on the basis of the change in the total number of colonies with different contamination levels (10^1 CFU/mL and 10^2 CFU/mL), atomization time (10, 20, 30, 40, and 50 s), atomizing distance (5, 10, 15, 20, 25, and 30 cm), and available chlorine concentration (ACC; 25.2, 30.2, 34.9, 40.5, 44.8, and 53.3 mg/L) as the main influencing factors. According to the relationship among flux, atomization area, and time, a kinetic model of SAEW atomization for the sterilization of tableware surfaces was established. The results indicated that the sterilization efficacy of SAEW gradually improved with decreased contamination levels (12.69 %–15.74 %), extended atomization time (13.68 %–46.58 %), and increased ACC (36.89 %–95.14 %). Based on the kinetics analysis, the change law of the kinetic model of SAEW atomization and sterilization of tableware surfaces with secondary pollution was found to be consistent with the change law of sterilization ($r^2 > 0.8$). The results of this study provide a theoretical basis for SAEW atomization for sterilization of secondary contaminated tableware surfaces and also contributes to the improvement of technological theory of SAEW sterilization.

1. Introduction

With the revival of the catering industry in the post-COVID-19 era, it has become particularly important to thoroughly disinfect tableware to reduce the risks of microbial infections [1]. In China, takeaway and dine-in options are both common. Since the COVID-19 epidemic, people have a stronger awareness of infection prevention and control, and pay close attention to the disinfection of tableware when using public tableware. However, the secondary microbial contamination [2] on disinfected tableware (10^1–10^3 CFU/mL) can easily be overlooked during storage and transportation [3], resulting in foodborne diseases. Although current methods of tableware disinfection including using electronic disinfection cabinets, chemical methods and boiling [4,5,6] have comparatively satisfactory disinfection efficacy, there are limitations such as operational difficulties and reagent residue during storage and transportation, which lead to the failure to completely solve the problem of secondary contamination [7]. Therefore, it is urgent to develop an efficient, simple, safe, and residue-free mild sterilization method for re-sterilization to solve the problem of secondary contamination on disinfected tableware.

Slightly acidic electrolyzed water (SAEW), also known as hypochlorous acid water, is a new type of green disinfectant characterized

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by high-efficient, rapid, broad-spectral, safe, and residue-free sterilization [8]. In 2002, it was recognized as a food additive (fungicide) by the Ministry of Health, Labor and Welfare of Japan [9], and it has been applied to the disinfection of food-processing utensils as well as fruits and vegetables. SAEW is also effective in eliminating the COVID-19 virus [10] and has no adverse effects on food ingredients, utensils, the environment, or humans [11]. In recent years, research on SAEW as a sustainable cleaning technology has mainly focused on its sterilization efficacy, sterilization mechanism, and storage conditions on food after sterilization [8,12–16]. However, the disinfection of tableware and food-processing utensils using atomized SAEW has been rarely reported [17,18]. The main methods for disinfection and sterilization using SAEW include cleaning, soaking, and atomization. Cleaning is effective for a short duration only, and soaking results in water stains. Nevertheless, it is worth noting that atomization has the advantages of simple operation, large atomization area, and no residue or water stains, so it has been widely used in various fields such as medicine [19], industry [20,21], and agriculture [22]. However, the application of SAEW atomization for sterilization of tableware has not been thoroughly researched yet.

In this study, SAEW subjected to atomization was applied to realize the disinfection of tableware surfaces and to study the change law of sterilization based on which the kinetic model of SAEW atomization for sterilization was analyzed. The corresponding results can provide a theoretical basis for the sterilization of tableware surfaces with secondary contamination.

2. Materials and methods

2.1. Materials and reagents

In this study, 304 food-grade stainless steel bowls (6.0 cm in height and 11.5 cm in diameter, from Deerlong Stainless Steel Tableware Factory, Jieyang City, Guangdong Province) were purchased from the farmer's market near Yunnan Agricultural University.

NaCl, $Na_2S_2O_3$, HCl, KI, glacial acetic acid, soluble starch, and analytical grade reagents were obtained from Tianjin Fengchuan Chemical Reagent Co., Ltd. Further, a nutrient broth and violet red bile lactose agar were purchased from Beijing Land Bridge Technology Co., Ltd (Chaoyang District, Beijing, China). In addition, *Escherichia coli* ATCC 25922 was obtained from China General Microbiological Culture Collection Center (Chaoyang District, Beijing, China).

2.2. Instruments and equipment

A HD-240 L "Shuishen" slightly acidic hypochlorous acid water generator (Shanghai Want Food Group Co., Ltd.), BS11OS electronic balance (Beijing Sartorius Instrument System Co., Ltd.), PL303 analytical balance and FE20 laboratory pH meter [Mettler-Toledo Instruments (Shanghai) Co., Ltd.], YXQ-SG41-280A high-pressure steam sterilizer (Shanghai Shengyin Medical Instrument Co., Ltd.), HPX-9272ME digital display electric heating incubator (Medical Equipment Factory of Shanghai Bosun Industrial Co., Ltd.), JJCJ-CJ-1FD ultra-clean workbench (Suzhou Jinjing Purifying Equipment Technology Co., Ltd.), ZD-85A dual-function digital display constant-temperature oscillator (Changzhou Langyue Instrument Manufacturing Co., Ltd.), HH-6 electric heating constant-temperature water bath (Changzhou Puda Scientific Instrument Co., Ltd.), and LY-9115 portable atomizer (Zhejiang Luyao Electronic Technology Co., Ltd.) were employed.

2.3. Experimental methods

2.3.1. Preparation of E. coli suspension

The lyophilized powder of *E. coli* was activated, cultured in a constant-temperature oscillator at 37 °C for 24 h, and stored in a refrigerator at 4 °C. The culture medium was changed every two weeks. The colonies were collected using an inoculation ring and transferred into a new broth medium placed in a constant-temperature oscillator and cultured at 37 °C for 24 h before each use. The operation was repeated three consecutive times so that the number of viable bacteria in the solution reached 10^8 CFU/mL.

2.3.2. Preparation of SAEW and determination of physical and chemical parameters

SAEW was generated using the "Shuishen" slightly acidic hypochlorous acid water generator with 6 % (v/v) hydrochloric acid solution prepared as an auxiliary solution and tap water as the raw water. The pH value and available chlorine concentration (ACC) of SAEW were measured before each experiment. Then, SAEW was placed into a portable atomizing spray bottle (The maximum atomization rate is 0.256 mL/min, the median ion diameter is 0.3–0.5 μm, the continuous spray time is more than 1 h, and the volume 35 mL) for use. The pH value and oxidation–reduction potential (ORP) were measured by a pH/ORP/conductivity comprehensive tester, and ACC was determined through iodometry.

2.3.3. Sterilization of tableware surface

2.3.3.1. Rapid disinfection of tableware with portable atomizer containing SAEW. The number of viable bacteria on the surface of some bowls simulated contamination was measured after treatment with sterile normal saline at room temperature (25 ± 2 °C). These were set as the control group and treated with different spray distances (5, 10, 15, 20, 25, and 30 cm), different ACC conditions (25.2, 30.2, 34.9, 40.5, 44.8, and 53.3 mg/L), and different atomization time (10, 20, 30, 40, and 50 s), and each experiment was repeated three

times. As shown in Fig. 1, the atomization area s (cm^2) can be calculated as follows:

$$s = \frac{r^2}{L^2} \tag{1}$$

2.3.4. Sampling and determination of E. coli count on tableware surfaces

The tableware was placed in a high-pressure steam sterilization pot at 121 °C. After sterilization for 15 min, the tableware was dried in an ultra-clean workbench. 1 mL bacterial suspension was added into the bowl evenly, and the bowl was rotated to fully contact the bacterial liquid for 30 min. After atomization and sterilization, the sterile filter paper was moistened with sterile physiological saline and attached to the inner surface of the tableware. The surface of the tableware was smeared and sampled. After 30 s, the filter paper was removed and immediately placed into a sterile tube containing 50 mL of sterile normal saline, followed by vigorous shaking using vortex mixer for about 20 s to obtain a stock solution.

The plate pouring method was adopted, where the samples were inoculated into a 90 mm-diameter culture dish and cultured in a 37 °C constant-temperature incubator for 18–24 h. Finally, the number of *E. coli* in the sample solution was calculated based on the average number of colonies in each group/treatment (n = 3) [23].

2.3.5. Statistical analysis

Origin 8.5 software (OriginLab Corp., USA) was used for graphing and fitting analysis, Excel 2010 (Microsoft Corp., USA) was applied for data processing, and Duncan comparison for significance analysis of SPSS 18.0 software (SPSS Inc., USA) was conducted. The results were expressed as mean ± standard deviation.

3. Results and discussion

3.1. Factors influencing atomized-SAEW sterilization efficacy

3.1.1. Influence of different contamination levels on sterilization efficacy

To investigate the effect of different pollution levels on the sterilization of the tableware surface, the concentrations of *E. coli* suspensions on the tableware surfaces were set at 10^1 CFU/mL and 10^2 CFU/mL to simulate contamination. Then, the number of viable bacteria on the tableware surfaces was detected under the conditions of ACC of 34.9 mg/L, atomizing distance of 10 cm, and different atomization times of 10, 20, and 30 s, with the sterile normal saline treatment as the control.

According to Fig. 2, different concentrations of bacterial suspensions were utilized to simulate different levels of pollution. With the increase of atomization time, the number of viable bacteria on the tableware surface evidently decreased. When the concentration was 10^2 CFU/mL, the number of viable bacteria on the tableware surface decreased to 1.93, 1.72, and 1.01 logCFU/mL after an atomization time of 10, 20, and 30 s, respectively. With the concentration of 10^1 CFU/mL, no bacteria were detected following 20 s of atomization time.

It has been revealed that the sterilization efficacy of SAEW is related to the concentration of the bacterial suspension: the higher the concentration of the bacterial suspension is, the longer the sterilization time will be (Rebezov et al., 2022). Consistent with this study, SAEW effectively reduced the population of *E. coli*, *Salmonella* spp. and *Staphylococcus aureus*, and it was found that both spray time and ACC had significant influences on the sterilization efficacy [24].

3.1.2. Influence of atomization time on tableware-sterilization efficacy

To investigate the influence of the atomization time on the sterilization efficacy in the case of *E. coli* contamination on tableware surfaces, the number of viable bacteria on the surfaces was determined under the conditions of ACC of 34.9 mg/L, atomizing distance

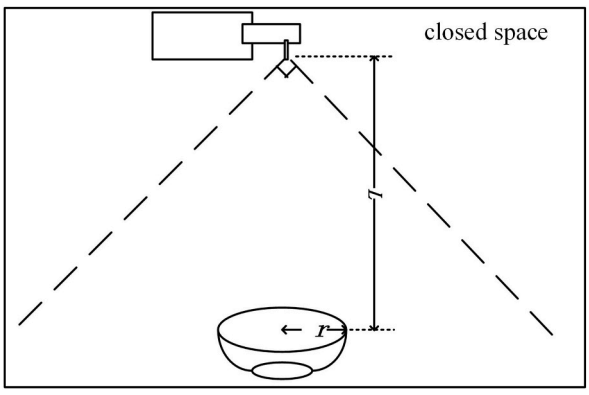

Fig. 1. Schematic diagram of disinfection of tableware using portable atomizer
Note: L is the atomizing distance, and r is the radius of the stainless steel bowl.

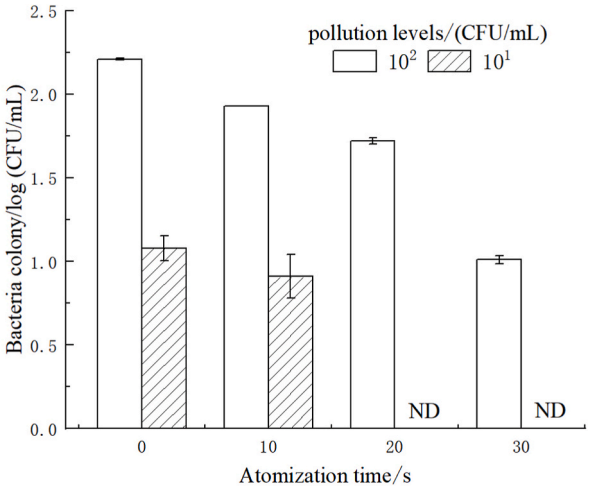

Fig. 2. Influence of different pollution levels on sterilization efficacy
Note: ND means "not detected."

of 10 cm, and different atomization times of 10, 20, 30, 40, and 50 s, with the sterile normal saline treatment as the control.

As shown in Fig. 3, the atomization time had a significant effect on the number of viable bacteria on the tableware surface. As the atomization time was increased, the number of viable bacteria on the tableware surface was gradually decreased. After different atomization time (10, 20, 30, 40, and 50 s), the number of viable bacteria on the tableware surface dropped from the initial 2.34 logCFU/mL to 2.02, 1.88, 1.89, 1.68, and 1.25 logCFU/mL, respectively. It is worth noting that the effect of SAEW on different harmful microorganisms is diverse, so the sterilization time of SAEW also varies [25,26].

3.1.3. Influence of spraying distance on tableware-sterilization efficacy

To investigate the influence of the atomizing distance on the sterilization efficacy in the case of *E. coli* contamination of tableware surfaces, the number of viable bacteria on the tableware surface was determined after atomization for 20 s with different atomizing distances of 5, 10, 15, 20, 25, and 30 cm when the ACC was 34.9 mg/L, with sterile normal saline treatment as the control.

The atomizing distance affected the tableware-sterilization efficacy (Fig. 4). With the increase in the atomizing distance, the number of viable bacteria on the tableware surface was finally reduced from the initial 2.34 logCFU/mL to 0.86, 1.68, 2.16, 2.11, 2.02, and 2.21 logCFU/mL, respectively. The number of viable bacteria on tableware surface increased significantly with the increase of atomization distance. Afterwards, as the atomization distance increased to 30 cm, the number of viable bacteria on the tableware surface fluctuated slightly. The optimal atomizing distance was determined to be 10 cm in view of the highest sterilization efficacy and the lowest energy consumption.

With the increase in the atomizing distance, the spray droplets from the atomizer showed undulating changes, and the number of deposited droplets may be affected by the size and drift of atomized particles. Moreover, the amount of liquid medicine deposited in a

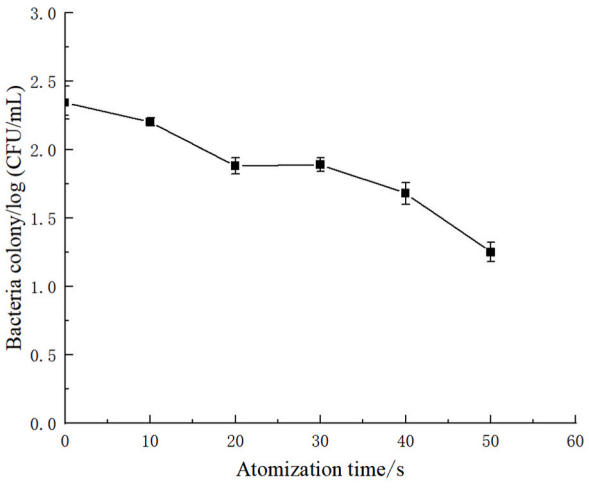

Fig. 3. Influence of atomization time on tableware-sterilization efficacy.

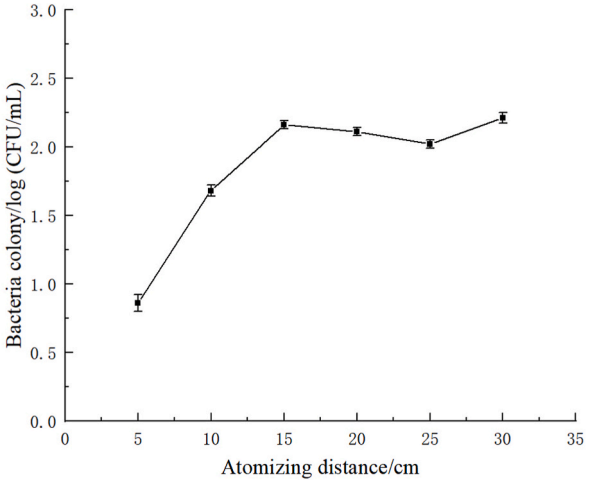

Fig. 4. Influence of atomizing distance on tableware-sterilization efficacy.

previous study had also presented a trend of first increasing and then decreasing with an increase in the atomizing distance [27], which is consistent with the results of this study.

3.1.4. Influence of ACC on tableware-sterilization efficacy

To achieve efficient, environmentally friendly, and residual-free disinfection, this requires adhering to the concept of the minimum dose and the optimal effect. Therefore, it is necessary to investigate the influence of different ACCs on the sterilization efficacy. The number of viable bacteria on the tableware surface was determined under the conditions of atomizing distance of 10 cm and different ACCs of 25.2, 30.2, 34.9, 40.5, 44.8, and 53.3 mg/L for 20 s, with the sterile normal saline treatment as the control.

The ACC of SAEW strongly affected the tableware-sterilization efficacy ($p < 0.05$) (Fig. 5). The number of viable bacteria on the tableware surface gradually decreased with the increase in ACC, which declined from the initial 2.06 logCFU/mL to 1.30, 0.92, 0.80, 0.26, and 0.10 logCFU/mL. In addition, a higher initial ACC of SAEW can lead to a stronger sterilization effect. Specifically, when the ACC was 30.2 mg/L, a good sterilization efficacy was realized after 20 s of atomization time, and the bacteria were not detected after 20 s when the ACC was 53.3 mg/L.

It has been demonstrated that SAEW atomization can effectively reduce the concentration of airborne microorganisms in a chicken coop [28], and a higher ACC and a greater spray volume resulted in higher purification efficacy [29]. Similarly, SAEW can inhibit the growth of microorganisms on the surface of tilapia fillets, and the sterilization efficacy improved with the increase of the available chlorine concentration of SAEW [30], which is basically consistent with the results of this study.

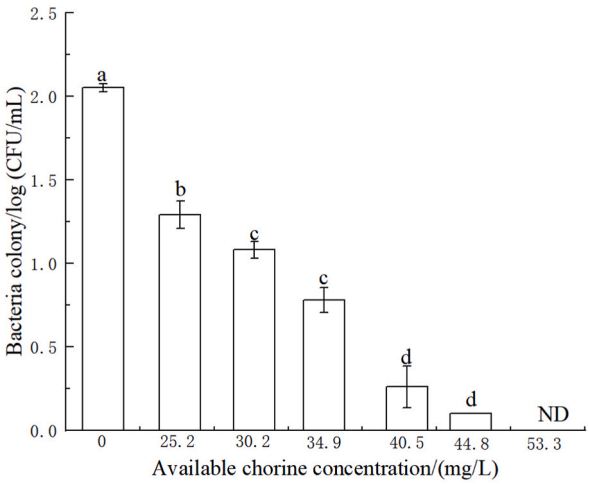

Fig. 5. Influence of ACC on tableware-sterilization efficacy
Note: Different letters indicate significant difference ($p < 0.05$), and ND stands for "not detected."

3.2. Kinetic model of tableware sterilization using atomized SAEW

3.2.1. Establishment of kinetic equation for tableware sterilization using atomized SAEW

A kinetic model of sterilization using the atomized SAEW was established on the basis of the relationship among flux, atomization area (Formula 1), and atomization time according to the formula below:

$$\frac{dQ}{dt} = Js, Q = Jst \tag{2}$$

Here, Q refers to the ACC (mg/L), J stands for the flux (after SAEW atomization, the amount flowing through a unit area per unit time is 0.35 mL/min), s indicates the atomization area (the contact area after atomization when the included angle of the atomizer nozzle is 90°, in cm^2), and t is the atomization time (s).

Based on the above formula, $-\frac{dQ}{dt} = KQN$ can be obtained, where K is the sterilization constant: $-\frac{dN}{dt} = KJstN$.

This equation can be converted into the following formula:

$$\frac{dN}{N} = -KJst \bullet dt \tag{3}$$

Therefore, an integral for the number of period sterilization could be derived:

$$\int_{N_0}^{N} \frac{dN}{N} = -\int_{0}^{t} KJst \bullet dt \tag{4}$$

The kinetic equation of the atomized-SAEW-based sterilization can be solved:

$$ln\frac{N}{N_0} = -KJst^2 \tag{5}$$

where N represents the number of viable microorganisms at any time after SAEW atomization (in CFU/mL), and N_0 is the initial number of microorganisms in CFU/mL.

3.2.2. Kinetic fitting analysis of tableware sterilization using atomized SAEW

At room temperature, the kinetic fitting results for atomized-SAEW-based sterilization of the tableware surfaces are shown in Fig. 6, where J was 0.35 mL/min and s was 135.86 cm^2. When the concentration of the bacterial suspension on the surface of the simulated tableware was 10^2 CFU/mL, no residual colonies were counted on the plate after 40 s of atomization time and enrichment culture. Therefore, the experimental data for atomization time of 10, 20, and 30 s were selected for fitting analysis. Similarly, when the concentration of the bacterial suspension on the surface of the tableware was 10^1 CFU/mL, the experimental data for the atomization time of 10 s were selected for fitting analysis, and the numerical values of parameters obtained from fitting are listed in Table 1.

The number of viable *E. coli* on the tableware surface after sterilization with atomized SAEW varied depending on the sterilization conditions (Fig. 6), including the original contamination level, atomization time, and ACC. Table 1 reveals that the coefficient of determination r^2 of the fitting analysis was greater than 0.8.

The first-order kinetic model not only predicts the texture changes and evaluates the edible period of food products during storage [31], but also reflects the change law of mass concentration ACC during the SAEW-based sterilization of tableware with *E. coli* [32]. Nowadays, kinetic models are widely used in the food industry, exerting a good predictive effect on food quality and sterilization process. However, the change law of sterilization for secondary pollution follows a relatively complex kinetic model, with few reported models and research on the kinetic law of atomized-SAEW-based sterilization of tableware. A kinetic model of sterilization using the atomized SAEW can provide a theoretical reference for controlling microorganisms on tableware surfaces. The kinetic model analyzed in this study can provide certain data support for the future application of atomized SAEW in tableware sterilization and food industry.

4. Conclusion

The change law of atomized-SAEW-based sterilization of tableware surfaces was investigated, and its kinetic model was established. SAEW had excellent sterilization efficacy. When the initial contamination level was kept constant, the sterilization efficacy improved with the prolongation of atomization time, the increase in ACC, and the decrease in the atomizing distance. Based on the relationship among flux, atomization area, and time, the kinetic model of SAEW atomization for the sterilization of tableware surfaces was constructed. The theoretical predicted values coincided with the actual measured values, and the fitting determination coefficients were all higher than 0.8, conforming to the change law of sterilization. This suggests that the model can predict the sterilization efficacy of atomized SAEW under different influencing factors, which can provide a theoretical reference for controlling microorganisms on tableware surfaces. Based on the current research conclusions, rapid tableware disinfection technology can be developed in the future to better solve the problem of secondary contamination of tableware.

Competing financial interests

The authors declare no competing financial interests.

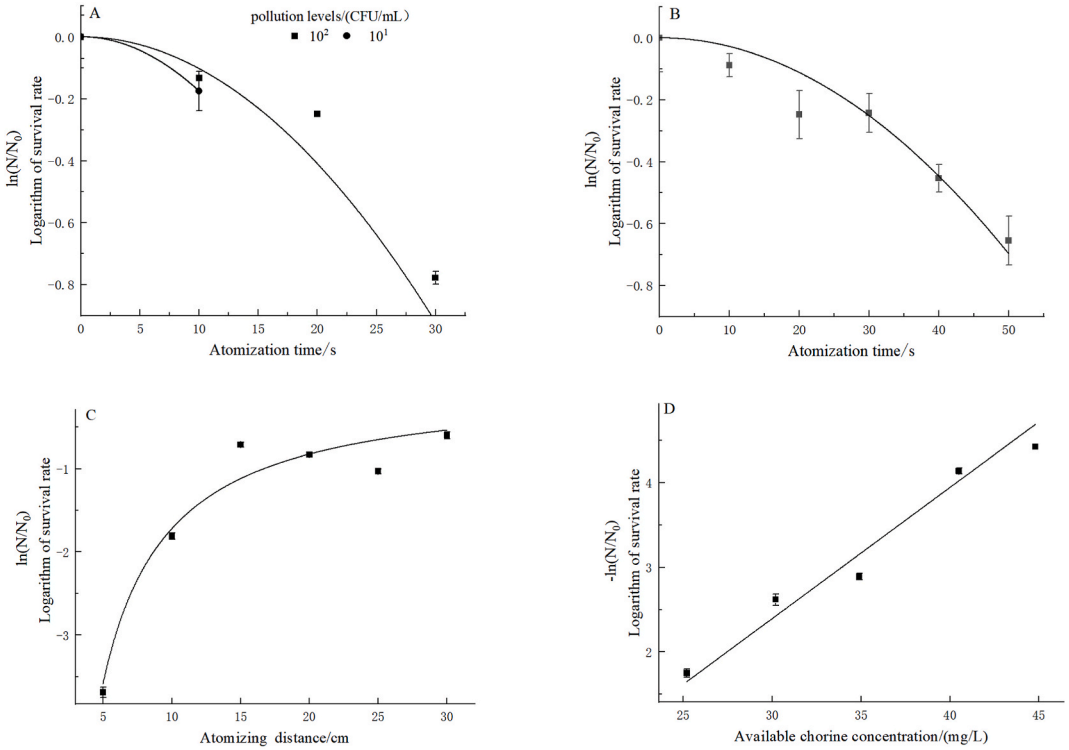

Fig. 6. Kinetic fitting of tableware sterilization using atomized SAEW
Note: Different pollution levels (A), atomization time (B), atomizing distances (C), and ACCs (D).

Table 1
Kinetic fitting parameters of tableware sterilization using atomized SAEW.

Influencing factor	Kinetic fitting parameter of atomizing sterilization	
	Sterilization constant K ($mL^{-1}\cdot cm^{-2}\cdot s^{-1}$)	Coefficient of determination r^2
Pollution level (10^1 CFU/mL)	0.014 ± 0.055	0.972
Pollution level (10^2 CFU/mL)	0.027 ± 0.098	0.987
Atomization time (s)	0.012 ± 0.068	0.893
Atomizing distance (cm)	0.004 ± 0.002	0.828
ACC (mg/L)	0.155 ± 0.019	0.945

Additional information

No additional information is available for this paper.

CRediT authorship contribution statement

Wanxin Zhao: Writing – original draft, Software, Formal analysis, Data curation. **Qing Gao:** Writing – original draft, Formal analysis, Data curation. **Yu Cao:** Writing – review & editing. **Yuanyan Meng:** Software, Methodology. **Jinsong He:** Writing – review & editing, Validation, Supervision, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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