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Inactivation efficiency of sodium hypochlorite on rotifers and rotifer eggs

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ABSTRACT

With the increasing application of ozone/biologically activated carbon (O_3/BAC) in drinking water treatment facilities, the excessive propagation and leakage of invertebrates have received increasing attention. Rotifers are commonly detected as the dominant invertebrate species in many areas. This study explored the inactivation efficiency of sodium hypochlorite on rotifers and rotifer eggs. The results show that the inactivation rate of rotifers increased with the chlorine dosage and contact time. The inactivation process can be divided into two phases: the lag phase and the inactivation phase. Temperature, pH and organic matter influence the inactivation rate of rotifers. Higher temperature and acidic conditions were conducive to inactivation of rotifers, but the inactivation rate decreased with an increase in organic matter concentration. The hatching ability of rotifer eggs weakened significantly when the eggs contacted sodium hypochlorite. However, rotifer eggs had weaker chlorine resistance than mature rotifers. To completely inactivate both rotifers and rotifer eggs, a contact time of at least 30 min with ≥ 1.5 mg/L chlorine is suggested. Based on the microscope and scanning electron microscope examination results, it is speculated that 1) sodium hypochlorite may have a toxic effect on the nerves and muscles of rotifers; and 2) sodium hypochlorite may destroy the armor of the rotifers and rotifer eggs. Once the armor is destroyed, sodium hypochlorite may enter the bodies through the damaged areas, which would accelerate the death of the rotifers and decrease the hatching ability of rotifer eggs.

Keywords: Rotifer; Sodium hypochlorite; Chlorine; Inactivation

1. Introduction

Technology that combines ozonation and biological activated carbon (O_3-BAC) has been applied for its efficient removal of dissolved organic matter [1,2], tastes and odors, micro cystin-LR, and disinfection by-product precursors [3]. As the running time of BAC filters increases, microorganisms may colonize the filters, which provide ideal living conditions [4]. These microorganisms include bacteria, protozoa, and metazoans (invertebrates). Invertebrates are discussed here. Invertebrates may penetrate the BAC filter and enter the filtered water because of their great vitality and transfer potential. Because this represents a hazard to drinking water safety [5], the leakage of invertebrates through BAC filters in O_3-BAC systems is a serious problem that has begun to attract researchers' attention.

Biological sampling of BAC filtrate between May 1994 and August 1995 at three different treatment plants along the Rhine river indicated that the BAC filters were colonized by invertebrates, with the dominant groups being rotifers and nematodes [6]. A survey of invertebrate colonization of BAC filters carried out weekly from October 2010 to December 2011 at a reservoir water treatment works in South China showed that the average invertebrate abundance in the filtrate was 12–18.7 times that of the pre-filtered water and that the dominant organisms were rotifers and copepods [7]. Another survey carried out in three waterworks along the lower reaches of the Yangtze River in China showed that rotifers, nematodes, tubifex worms and crustaceans were the dominant species [5]. The excessive multiplication of inver-

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tebrates also occurred in a pilot-scale BAC system in a water plant alongside the Dongjiang River, South China, and rotifers were also the dominant species [8]. Thus, rotifers have been commonly detected as the dominant species in many studies. Rotifers are known to ingest Cryptosporidium and Giardia (oo)cysts under laboratory conditions, and they were thought by Bichai et al. [9] to be the main potential predator of cysts and oocysts. The pathogens predated by rotifers can be transferred in drinking water because rotifers are suspected to lack the enzymes needed to digest the (oo) cysts [10]. In addition, some rotifers are harmful to human health. A case report of infection of the human urinary system by Lecane inermis Bryce was presented by Tan in 2000 [11]. Therefore, rotifers have the potential to harm human health. In view of the two reasons (large proportion and the potential threat to safe drinking water), the removal of rotifers in Drinking Water Treatment Plants (DWTPs) is vital.

Based on our previous research, both rotifers and rotifer eggs can penetrate BAC filters, therefore, the disinfection stage is the final line of defense. Chlorine is the most commonly used chemical method of disinfection [12], and hypochlorite, especially sodium hypochlorite, is one of the most common disinfectants used in the chlorine disinfection process. Sodium hypochlorite can efficiently inactivate most microorganisms, including bacteria [13], fungi [14], viruses [15] ,and parasites [16]. However, there are almost no reports of the inactivation of rotifers by sodium hypochlorite.

Therefore, the objectives of this paper are to (i) evaluate the efficiency of the inactivation of rotifers and rotifer eggs by existing disinfection technology, and (ii) propose disinfection strategy which takes invertebrates into account.

2. Methods and materials

2.1. Culture of rotifers

The rotifers used for the experiments were the common freshwater rotifer *Brachionus calyciflorus* Pallas cultured on *Chlorella* in high density [17]. Rotifers were cultured in a 1-L conical tank at 25°C ± 1°C on a photo period cycle of 16:8 h light: dark at 200 lux in an incubator. The culture medium contained EPA (96 mg $NaHCO_3$, 60 mg $CaSO_4$, 60 mg $MgSO_4$, 4 mg KCl in 1 L deionized water with the pH adjusted to 7.5) and *Chlorella* (cell density around 10^6 cells ml^{-1}). The culture medium was refreshed every 24 h.

2.2. Chlorine treatment of rotifers

In the experiments, 28–32 adult, active individuals of similar size, which were isolated from the same sources were collected in culture dishes (60 mm in diameter). Then 10 mL of deionized water was added to the culture dishes. The initial pH (detected by Multi 9630 IDS, WTW, Germany) was adjusted with sodium hydroxide and sulfuric acid; the temperature was controlled with an incubator (GZX-150BS-III, CIMO, China); and the initial organic matter concentration was prepared by the dilution of a stock solution of humic acid (detected by Element liqui TOC-L CPH CN200, SHIMADZU, Japan). Solutions containing 100 mg/L available chlorine were prepared from commercially available sodium hypochlorite (available chlorine ≥ 5.5%) and were then added to the culture dishes to provide reaction mixtures containing 0.5, 1.0, 1.5, and 2.0 mg/L available chlorine. After 2, 5, 10, 15, 20, 25, 30, 45, 60, 75, 90, 105, and 120 min of treatment, residual free chlorine was neutralized by the immediate addition of sodium thiosulphate, and the rotifers were then observed under a stereo microscope (SZ61TR, Olympus, Japan). A rotifer was regarded as dead if it made no observable response when grasped with a pair of fine forceps. Triplicate observations were carried out for each chlorine concentration.

The inactivation rate of rotifers was calculated as follows.

$$\text{Inactivation rate} = \frac{(N_0 - N_t)}{N_0} \times 100\% \quad (1)$$

where N_0 is the initial living rotifer density at the start of the experiment and N_t is the residual living rotifer density at the time t.

Duplicates of each chlorine concentration were made at the same time. After 15, 30, 45, 60, 75, 90, 105, and 120 min of treatment, residual available chlorine was immediately detected with a portable residual chlorine comparator (HACH, USA).

2.3. Chlorine treatment of rotifer eggs

The apparatus and treatment protocol for rotifer eggs was as above. After contact times of 2, 5, 10, 15, 30, and 60 min, the eggs were transferred to a culture dish containing culture medium and cultured in incubator at 25°C. The hatching rate of the eggs was observed and recorded every 12 h, which was defined as complete separation of the larvae from the egg. Eggs in the control group were counted in the same way but without chlorine treatment.

The hatching rate of rotifer eggs was calculated as follows.

$$\text{Hatching rate} = \frac{(n_0 - n_t)}{n_0} \times 100\% \quad (2)$$

where n_0 is the initial number of rotifer eggs at the start of the experiment and n_t is the number of rotifer eggs that had changed to larvae at time t.

2.4. Observation of changes to rotifer morphology

2.4.1. Microscopic examination

Duplicate dosages of chlorine of 1.0 mg/L were made. The motion states of rotifers were observed using a biological microscope (BX-51, Olympus, Japan), and photographs of the rotifers were taken at contact times of 2, 5, 10, 15, 20, 25, 30, 45, and 60 min until the rotifers were dead.

2.4.2. Scanning electron microscope (SEM) examination

A scanning electron microscope (Hitachi S-3400) was used to investigate the body structure changes of the rotifers before and after chlorination (chlorine dosage 1.0 mg/L, contact time 60 min). Sampled individuals were fixed with 2.5% glutaraldehyde and washed in a sterile phosphate

buffer, followed by dehydration in an ethanol series, critical point drying, and coating with gold.

3. Results and discussion

3.1. Available chlorine changes

Fig. 1 shows the change in available chlorine with contact time during the inactivation process. The available chlorine concentration reduced quickly in the first 15 min and then constantly decreased as the contact time increased, but the rate of decrease slowed. After 120 min contact, the maximum reduction of the available chlorine was only 0.24 mg/L and the maximum reduction rate was only 26.5% compared with the initial chlorine concentration. It was concluded that the chlorine consumption during the inactivation of rotifers was very low in this experiment.

In 1978, Johnson et al. proposed a first-order kinetics of chlorine consumption [18]:

$$C_t = C_0 exp(-kt) \quad (3)$$

where C_t (mg/L) is the available chlorine concentration at time t (min), C_0 (mg/L) is the initial chlorine concentration, t is the reaction time and k is the first-order reaction rate constant (min^{-1}).

The first-order kinetics fitting of the chlorine consumption data was achieved by setting ln (C_t/C_0) as the ordinate and t as the abscissa. The fitting results showed that the model is a good fit for the chlorine consumption in this experiment and the correlation coefficients (R^2) all reached 0.90. There is a prominent linear relation between ln (C_t/C_0) and t, indicating that the chlorine consumption during the inactivation of the rotifers fitted the first-order kinetics model.

3.2. Inactivation effect on rotifers

The experimental results for the inactivation of rotifers with sodium hypochlorite at 20°C and pH 7.5 are presented in Fig. 2. The inactivation rates were averaged and are indicated by error bars.

As is shown, at each chlorine concentration, there was a lag phase at the beginning of the inactivation. In the lag phase, t_{lag}, no rotifers were inactivated. When the initial chlorine dosages were 0.5 mg/L, 1.0 mg/L, 1.5 mg/L, and 2.0 mg/L, t_{lag} was 30 min, 15 min, 10 min, and 5 min, respectively. After the lag phase, the rotifers were gradually inactivated. For the tested rotifers, 100% inactivation rates were achieved within 30 min when the initial chlorine dosages were 1.5 and 2.0 mg/L. However, at the same contact time of 30 min, the inactivation rate was only 14.8% when the initial chlorine dosage was 1.0 mg/L, and another 60 min was needed for completely inactivation. When the initial chlorine dosage was 0.5 mg/L, the inactivation rate was 89.0% at the end of the experiment. Therefore, after the lag phase, the inactivation rate of rotifers increased with increasing chlorine dosage and extended contact time until inactivation was complete.

Yan et al. [19] took *Plectus* sp., a nematode, as the research object of an inactivation experiment using sodium hypochlorite. Their results showed that with a chlorine dosage of 12.0 mg/L and a contact time of 30 min, the inactivation rate was only about 3.3%. Zhang et al. [20] took copepods as the research object and found that with a chlorine dosage of 2.0 mg/L and a contact time of 30 min, the inactivation rate was about 70%. Thus, it can be concluded that nematodes have the strongest chlorine resistance and that rotifers can be inactivated the most easily among these three types of invertebrate.

3.3. Effects of temperature, pH and organic matter on the inactivation rate of rotifers

3.3.1. Temperature

The effects of temperature on the inactivation rate are shown in Fig. 3. The temperature was controlled at 10°C, 20°C, and 30°C. The inactivation of rotifers at lower temperature required a longer contact time to obtain the same inactivation rate as at higher temperature. In other words, increasing the temperature can improve the inactivation efficiency. It has been suggested that sodium hypochlorite disinfection mainly depends on the hypochlorous acid

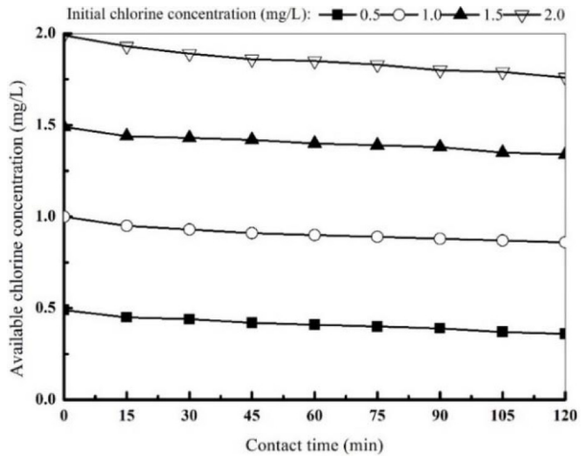

Fig. 1. Available chlorine changes with contact time during the rotifer inactivation process.

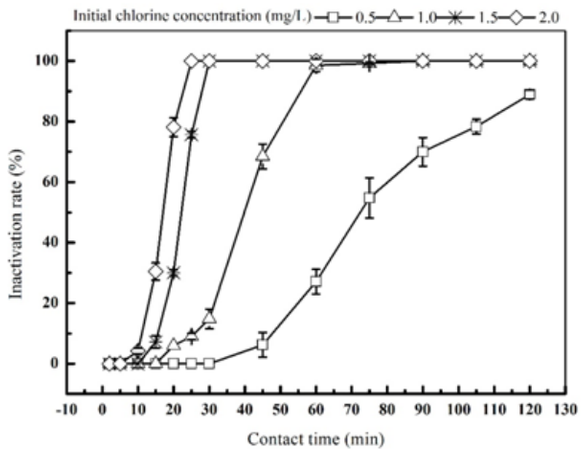

Fig. 2. Inactivation rates for rotifers at various chlorine concentrations in relation to contact time.

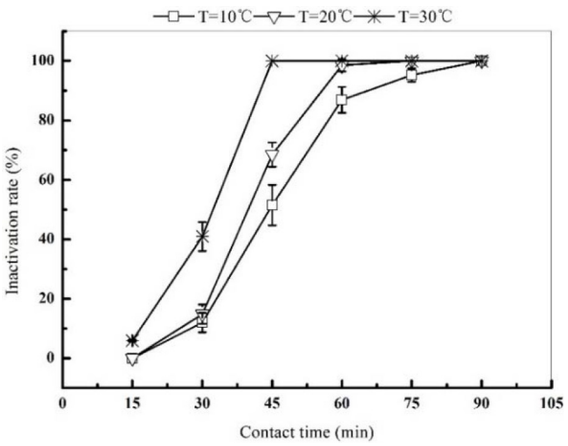

Fig. 3. Effect of temperature on inactivation rate of rotifers (pH 7.5, chlorine dosage of 1.0 mg/L, free of organic matter).

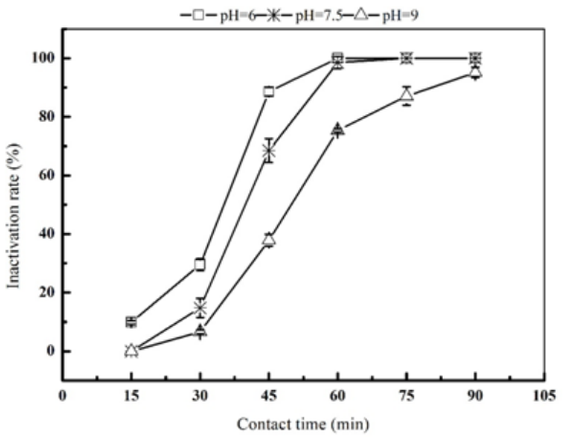

Fig. 4. Effect of pH on inactivation rate of rotifers (chlorine dosage of 1.0 mg/L, temperature of 20°C, free of organic matter).

generated during the hydrolysis of sodium hypochlorite [21]. Because hydrolysis is an endothermic reaction, a temperature increase is conducive to the reaction. In addition, studies have shown that as the temperature rises, the decomposition rate of sodium hypochlorite is accelerated [22]. When the temperature is below 25°C, the decomposition is slow; when the temperature is higher than 30°C, the decomposition speeds up significantly. Therefore, in this experiment, when the temperature was about 30°C, the inactivation rate of rotifers increased significantly. Fig. 3 shows that after 45 min contact time, the rotifers were completely inactivated at 30°C. The inactivation rates were 51.5% and 68.5%, respectively, at 10°C and 20°C. As these three kinds of temperature, 10°C, 20°C, and 30°C could respectively represent the winter, spring and autumn, and summer in the lower reaches of the Yangtze River, it was suggested that in DWTPs the chlorine dosage or the contact time could be increased properly in winter.

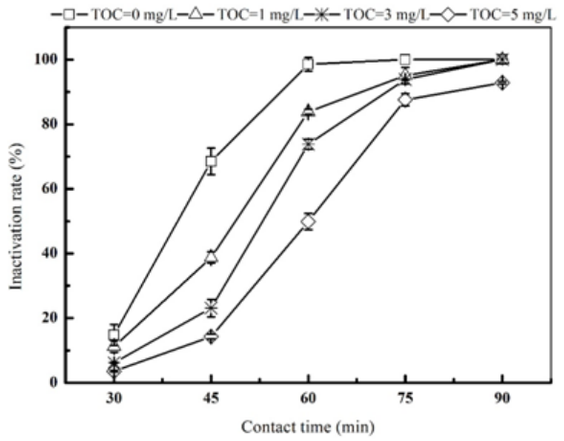

Fig. 5. Effect of organic matter on inactivation rate of rotifers (pH 7.5, chlorine dosage of 1.0 mg/L, temperature of 20°C).

3.3.2. pH

The effects of pH on the inactivation rate are shown in Fig. 4. The initial pH was controlled at 6, 7.5, and 9. As is shown, pH had great effects on the inactivation rate of rotifers. Compared with neutral conditions, acidic conditions were benefit to the inactivation of rotifers by sodium hypochlorite, while alkaline conditions weakened the inactivation efficiency. It was analyzed [23,24] that sodium hypochlorite hydrolyses in water and exists mainly in two forms, hypochlorous acid (HOCl) and hypochlorite ions ($^{-}$OCl). And the proportion of the two forms depends on the pH of the solution. HOCl predominantly exists at low pH levels of 4–6, while $^{-}$OCl at pH levels of 8.5–10. And HOCl has been suggested to have an antimicrobial effect around 80–100 times stronger than $^{-}$OCl [25].

3.3.3. Organic matter

The effects of organic matter on the inactivation rate are shown in Fig. 5. The organic matter concentration was controlled at four levels: 0 mg/L (control), 1 mg/L, 3 mg/L, and 5 mg/L. As is shown, the inactivation rate of rotifers decreased with increasing organic matter concentration. As a reductive substance, organic matter may react with sodium hypochlorite and decrease the available dose of chlorine for the inactivation of rotifers. Therefore, competition between the organic matter and rotifers resulted in a decrease in the inactivation rate. To enhance the inactivation of the invertebrates, it was suggested that in DWTPs the organic matter concentration in the water before disinfection should be controlled as low as possible.

3.4. Effect on the hatching rate of rotifer eggs

Because eggs are smaller than adults, they can penetrate the BAC filters and enter the treated water more easily. Therefore, it is also very important to investigate the inactivation of rotifer eggs by sodium hypochlorite.

Fig. 6 shows the hatching rates for rotifer eggs at various chlorine concentrations. At each chlorine concentration, t = 0 min was the control group. In the control group, the

hatching rate reached 74.6% after 6 h of culture and 100% of the rotifer eggs were hatched within 24 h. The result showed that [26], the hatching time of rotifer eggs shortened with the increase of temperature. When the temperature was 25°C, the average hatching time of rotifer eggs was 11.5 h. In this study, under the hatching temperature of 25°C, 98.4% of the eggs hatched out within 12 h culture, which was in agreement with the results obtained in the literature.

When the eggs contacted sodium hypochlorite, the hatching ability weakened significantly. However, the hatching time of rotifer eggs had not been changed obviously. As Fig. 6 shows, the hatching rate under different chlorine dosage and different contact time tended to become equilibrium after 12 h hatching. Therefore, the hatching rates within 12 h hatching were discussed in the following discussion.

When the initial chlorine dosage was 0.5 mg/L and the contact time was 2 min, the hatching rate of rotifer eggs decreased to 67.8%; when the contact time was 15 min, the hatching rate decreased under 10%; and the rotifer eggs lost their hatching ability completely after the contact time of 60 min. When the initial chlorine dosage was 1.0 mg/L and the contact time was 2 min, the hatching rate of rotifer eggs decreased to 51.7%; when the contact time was 10 min, the hatching rate decreased under 10%; and the rotifer eggs lost their hatching ability completely after the contact time of 30 min. When the initial chlorine dosage was 1.5 mg/L and the contact time was 2 min, the hatching rate of rotifer eggs decreased to 22.9%; when the contact time was 5 min, the hatching rate decreased under 10%; and the rotifer eggs lost their hatching ability completely after the contact time of 10 min. When the initial chlorine dosage was 2.0 mg/L and the contact time was 2 min, the hatching rate decreased under 10%, which was only 9.4%; and the rotifer eggs lost their hatching ability completely after the contact time of 5 min. Therefore, when the contact time was the same, the hatching ability weakened as the chlorine dosage increased; when the chlorine dosage was the same, the hatching ability weakened as the contact time extended. And the time when the rotifer eggs lost their hatching ability completely shortened as the increase of the chlorine dosage.

Table 1 shows the inactivation rates of adult rotifers and the hatching rates of rotifer eggs at the same chlorine dosages and contact times. It can be concluded that the adult rotifers had stronger chlorine resistance than did the rotifer eggs. In addition, when the chlorine dosage was ≥ 1.5 mg/L and the contact time was ≥30 min, both rotifers and rotifer eggs could be inactivated completely. Therefore, 1.5 to 2.0 mg/L chlorine disinfectant and at least 30 min contact time are suggested for DWTPs.

Table 1
Comparison of chlorine inactivation of adult rotifers and rotifer eggs

Available chlorine concentration (mg/L)	Contact time (min)		
	15	30	60
0.5	0/9.70%	0/3.05%	27.10%/0
1.0	0/3.50%	14.80%/0	98.55%/0
1.5	7.15%/0	100%/0	100%/0
2.0	30.45%/0	100%/0	100%/0

Note: inactivation rate of rotifers/hatching rate of rotifer eggs

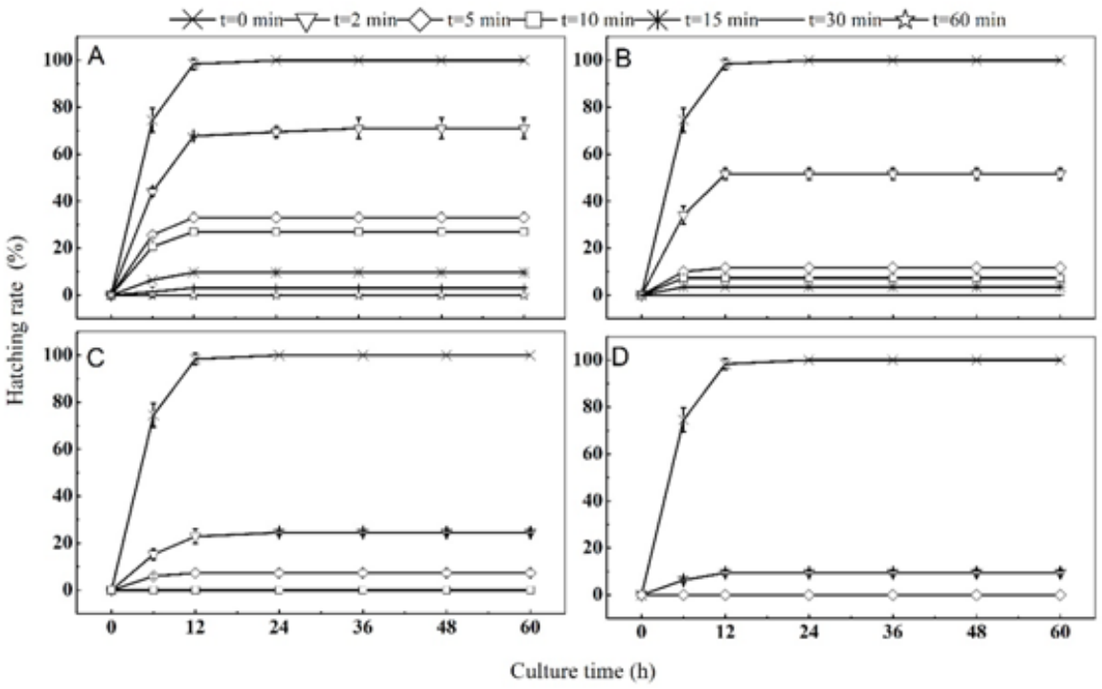

Fig. 6. Hatching rates for rotifer eggs at various chlorine concentrations (A: 0.5 mg/L; B: 1.0 mg/L; C: 1.5 mg/L; D: 2.0 mg/L).

3.5. Effect of sodium hypochlorite on rotifer morphology

3.5.1. Microscopic examination

A suit of morphological features was examined microscopically along with the contact time when the chlorine dosage was 1.0 mg/L. Before treatment with chlorine, rotifers swam around in the culture dish with their ciliary rings rotating quickly. Once chlorine was added, their swimming ability weakened significantly. About two minutes later, the rotifers rested on the bottom of the culture dish and rotated in place. During this time, the rotifers could be divided into two parts: the head region of one part of the rotifers stretched back and forth in vivo and in vitro; head region of the other part retracted into the body completely, the so-called 'armor model'. Then, about 5 min later, almost all the rotifers came into the armor model. After a contact time of about 25 min, the head region of the rotifers gradually stretched out of the bodies until it was completely stretched out. Finally, the rotifers gradually died. All the rotifers in this experiment experienced the same morphological changes after being treated with chlorine. A rotifer was selected randomly and photographed at each contact time. Fig. 7 illustrates the morphological changes.

The movement of rotifers is controlled by two nerves inserted in the infraciliature and is realized by the rotation of ciliary rings on the head. The changes in the swing ability of the ciliary rings and the nonrestrictive shrinking of the inner neuromusculature could result in changes to the rotifers' swimming ability [27]. Because contact with chlorine weakened the rotifers' swimming ability rapidly and significantly, it is speculated that sodium hypochlorite may have a toxic effect on their nerves and muscles.

3.5.2. SEM examination

A suite of morphological features was examined under SEM before and after inactivation. In the control group, almost all individuals were in the armor model, and most of the rotifers and their eggs presented no damage except wizening (Figs. 8 A–D). However, after treatment with 1.0 mg/L chlorine, almost all the head region of the individuals was stretched out of the bodies (Figs. 8 E–H), which was the same as the observation under the microscope. In addition, the armor part of the rotifers and their eggs were damaged, and the inner substances were observed. Therefore, it is speculated that sodium hypochlorite may destroy the armor of the rotifers and rotifer eggs. Once the armor is destroyed, sodium hypochlorite may enter the bodies through the damaged area, which would accelerate the death of the rotifers and the loss of hatching ability of rotifer eggs.

4. Conclusions

(1) Sodium hypochlorite can inactivate both rotifers and rotifer eggs efficiently. Rotifers had stronger chlorine resistance than rotifer eggs. To completely inactivate both adult rotifers and rotifer eggs, a chlorine dosage of at least 1.5 mg/L and a contact time of at least 30 min are suggested.

(2) Temperature, pH and organic matter affect rotifer inactivation. The inactivation rate increased with the temperature increase and decreased as the organic matter concentration increased. Acidic conditions were benefit to the inactivation of rotifers, while alkaline conditions weakened the inactivation efficiency. To guarantee the inactivation efficiency, it was suggested that in DWTPs the chlorine dosage or the contact time could be increased properly in winter and the organic matter concentration in the water before disinfection should be controlled as low as possible.

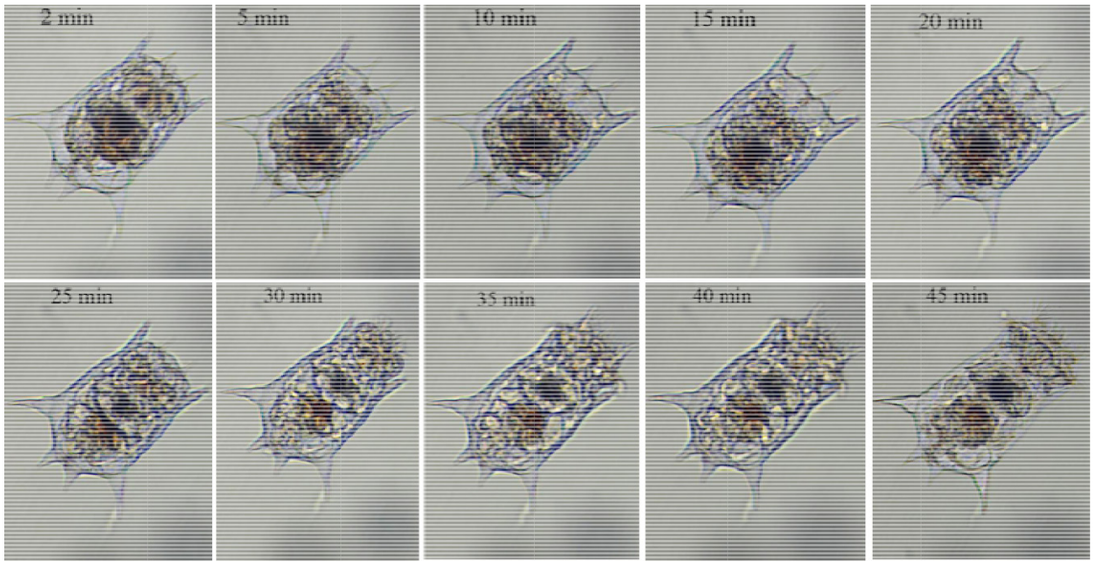

Fig. 7. Rotifer morphology changes with the contact time as seen under biological microscope.

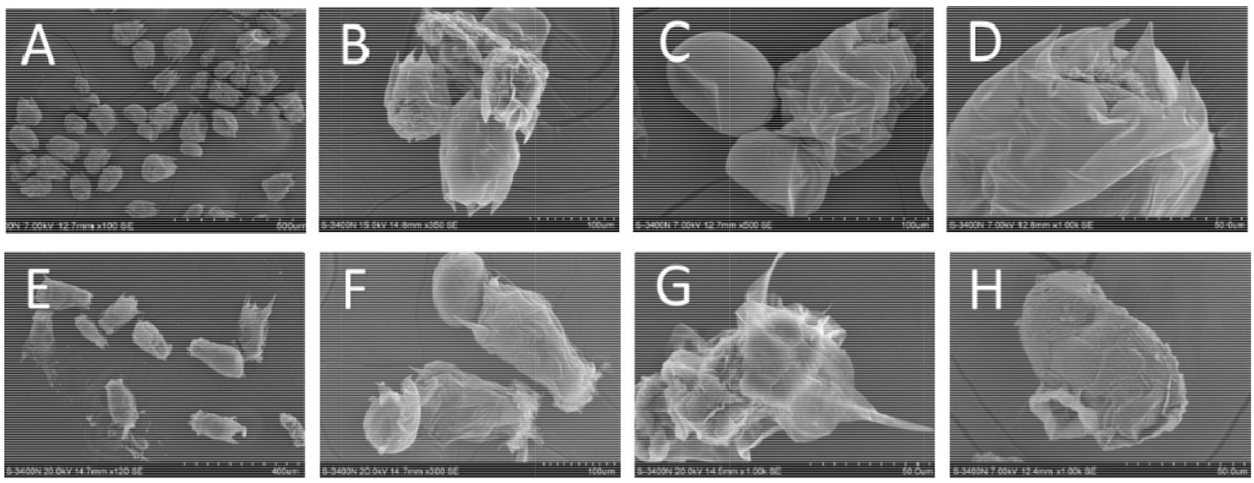

Fig. 8. Rotifer morphology under SEM (A–D: control group; E–H inactivation for 60 min).

(3) The nerves, muscles, and armor of rotifers and rotifer eggs may be destroyed by sodium hypochlorite during inactivation, resulting in the death of rotifers and the loss of hatching ability of rotifer eggs.
(4) As rotifers are usually the dominant species of invertebrates, the efficient inactivation of rotifers and rotifer eggs could significantly decrease the living invertebrate abundance and the reproduction of the invertebrates in the product water, which could greatly reduce the harm to safe drinking water supply.

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