Excess propagation and disinfection control of Copepod in an ozone-granular activated carbon filter in southern China

T. Lin, W. Chen and L. L. Wang

ABSTRACT

The reasons for the excess propagation of Copepod in an ozone-granular activated carbon filter were investigated and batch experiments were conducted to analyze the influences of CT value, pH and organic matter on the efficiency of disinfection of Copepod using chlorine and chloramines. The experimental results revealed that Copepod accounted for 70% of the zooplankton in the sand filter effluent that flowed into the ozone contact tank. Complete inactivation of Copepod was attained when the ozone dosage was 6 mg/l for 15 min contact time. The high bromide concentration in the raw water prevented increasing the ozone dosage due to the formation of bromate during ozone oxidation. Live Copepod entered the GAC filter via the ozone contact tank effluent. The average period of Copepod growth was approximately 8 days when cultured in the GAC filter water at 25°C and the survival ratio of the Copepod nauplii was greater than 80%. The number of Copepod in the GAC filter effluent was three times greater than the survival in the sand filter. Varying the pH from 6 to10 did not influence the disinfection efficiency of chloramines and the organic matter had rather low negative effects on the inactivation rate.

Key words | copepod of zooplankton, disinfection, excess propagation, GAC filter

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INTRODUCTION

In recent years, granular activated carbon (GAC) filters have been used as a method of advanced treatment for drinking water purification in some waterworks in southern China. The GAC filtration is usually applied following sand filtration and prior to final chlorination. However, some reports have indicated that the propagation of invertebrates can occur in GAC filters, thereby endangering the quality of the final product (Schreiber *et al.* 1997; Castaldelli *et al.* 2005; Weeks *et al.* 2007). Indeed, Copepods such as Cyclopoida and Harpacticoida are commonly found in GAC filters in waterworks in southern China (Li *et al.* 2007). Zooplankton has strong resistance against oxidation and is not effectively removed by conventional disinfection methods such as chlorination (Lin *et al.* 2004; Liu *et al.* 2007). In addition, the motility of zooplankton enables them to easily penetrate the GAC filter and enter the clear water reservoir of waterworks, and may even enter the municipal distribution network. Zooplankton can be seen by the naked eye, which would cause consumers to feel that the water is not sanitary. Furthermore, zooplankton may transmit pathogens, such as schistosome and eelworms; therefore, they have the potential to threaten human health (Wolrnarans *et al.* 2005; Bichai *et al.* 2008).

Drinking water purification by the ozone-granular activated carbon process does not enable the complete removal of Copepod in the ozone contact tank. Therefore, the effluent from the contact tank, which enters

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the GAC filter, often contains Copepod. As a result, propagation of Copepod is commonly observed on GAC filters. However, most studies of these systems that have been conducted to date have focused on the removal of micro-pollutants, and few have reported on the health risk of zooplankton. It is difficult to kill Copepod using liquid chlorine at dosages commonly used by drinking water utilities. Additionally, disinfection byproducts may be created when higher chlorine dosage is used to completely inactivate Copepod. As a substitute or a supplemental disinfectant for chlorine, chloramines produce fewer disinfection byproducts and have a longer exposure time than chlorine (Vasquez *et al.* 2006; Hua & Reckhow 2007). In addition, it has been reported that chloramines are more effective at inactivating Copepod than chlorine (Farrell *et al.* 2001). In this study, experiments were conducted to investigate the reasons for the propagation of Copepod in the GAC filters and to compare their disinfection by chloramines to disinfection with liquid chlorine.

MATERIALS AND METHODS

Culture of Copepod: mature Copepods were initially collected from GAC effluent in a municipal waterworks in southern China (Jiangsu Province) and then artificially cultivated in the laboratory. To ensure the accuracy of analysis, four parallel experiments were conducted during culture. In each experiment, five pairs of Copepod were cultivated in an aerated glass container with a volume of 5 l. To investigate the growth of Copepod in different water environments, water samples containing no Copepod were collected from the sand filter and GAC filter respectively. The water was then placed in a glass container with 5 cm of quartzite sand or GAC media at the bottom. Next, the glass container was incubated at a constant temperature (25°C) and photoperiod (16 h light/8 h dark), while the level of dissolved oxygen in the container was controlled at $7-10\,mg\,l^{-1}$ to simulate the conditions of the filter. The periods of Copepod growth were then investigated by measuring the growth interval among ovum, nauplii and adults. The clearance and feeding rates of the Copepod were analyzed using previously described methods (Strathmann 1967).

Disinfection experiment: to obtain homogenous samples (size and age), Copepod from the same generation was transferred from the culture container into 2 l glass reactors filled with water. To investigate the influences of organic matter in the water on the inactivation rate, natural organic matter (NOM) in the water source served as the total organic carbon (TOC) source (International Humic Substances Society (IHSS), Golden-Colorado). Briefly, dried powdered NOM was suspended in Millipore water (Milli-Q system; Millipore Corp., USA) to prepare a stock solution of 100 mg/l as TOC. Aliquots of the stock solution were then added to the reactor water to give final concentrations of 2, 4 or 6 mg/l. The organic carbon concentrations were confirmed using a Sievers 800 Total Organic Carbon (TOC) analyzer (Ionics Sievers, Boulder, CO).

To evaluate the influences of pH on the inactivation rate, the experiments were conducted in $0.01\,mol\,l^{-1}$ buffer solutions prepared by adding reagent grade phosphates, borate or carbonates to distilled water. Specifically, phosphates were used for experiments conducted at pH 6 and 8, while borate and carbonates were used to prepare buffering solutions with a pH of 10. The distilled water was prepared as described in the *Standard Methods* (APHA 1998).

A stock solution of chlorine was prepared by diluting a commercial solution of sodium hypochlorite (NaOCl, 9% active chlorine). The chlorine concentration was determined based on the free valid chlorine and the chloramine concentration was determined based on the combined valid chlorine (the ratio of chlorine to ammonia is 4:1 for the primary product of NH_2Cl). The number of Copepod was controlled at $10\,ind\,l^{-1}$ during the experiment to simulate the levels observed during the water treatment process. Individual Copepod were regarded as dead if they did not make at least one response when being stimulated with a pair of fine forceps. The inactivation rate was evaluated by the microscopic count method, with the number of dead Copepod being confirmed after disinfection.

Bromate detection: five parallel samples were evaluated for the presence of bromate using an ISC-1000 Ion Chromatography system (Dionex, USA).

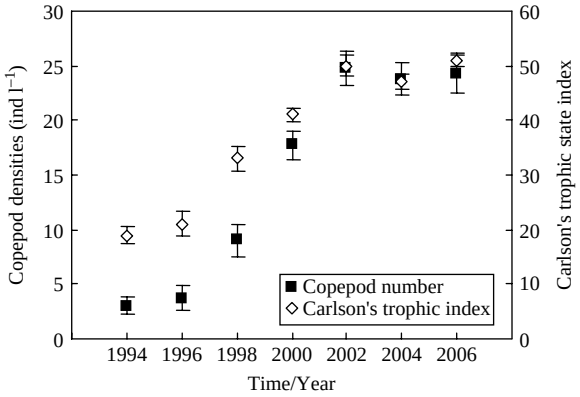

Figure 1 | Annual change of Copepod densities and Carlson's trophic state index in the water source ($n = 3$, $P < 0.05$).

THMs examination: five parallel samples were analyzed for the presence of THMs using an Agilent 7890A gas chromatography spectrometer (Agilent, USA).

RESULTS AND DISCUSSION

Annual change in Copepod densities in water source and during the treatment process

The annual change in Copepod densities in the water source is shown in Figure 1. The Copepod densities gradually increased as the eutrophic status of the water source increased (as indicted by the Carlson's trophic state index). The increasing number of Copepod in the water source provides the possibility for its presence in waterworks. The composition of the zooplankton was also investigated in the effluent of different treatment processes and the results are shown in Table 1.

As shown in Table 1, protozoa dominated the zooplankton population in raw water, followed by rotifers, while Copepod comprised only about 5% of the total zooplankton. However, most of the other zooplankton was likely removed by sand filtration, whereas the motility of Copepod enabled its entry into the subsequent ozone contact tank. As a result, the Copepod comprised approximately 70% of the zooplankton in the effluent of the sand filter. As shown in Figure. 2, Copepod was detected in the effluent of the ozone contact tank. These findings indicate that it was not completely eradicated by ozone oxidation under the water treatment conditions, which consist of 15 min contact time and an ozone dosage of 3.0 mg l^{-1}. As a result, Copepod and ozone contact tank effluent enter the subsequent GAC filter. The densities of Copepod in the GAC filter effluent were approximately three times higher than that of the sand filter (Figure 2), which indicates that the propagation of Copepod occurs in the GAC filter. Therefore, the inactivation rate and influencing factors of Copepod removal were investigated during ozone treatment.

Inactivation rate and influencing factors of Copepod removal by ozone oxidation

Water samples were collected from the influent of the ozone contact tank and then subjected to treatment with various dosages of ozone at 25°C, TOC 4.5 mg l^{-1} and pH 6.89 (Figure 3). The inactivation rate of Copepod gradually increased as the ozone dosage and the contact time increased, with complete removal of Copepod occurring when the ozone dosage was 6 mg l^{-1} and the contact time was 15 min.

Copepod has a body surface consisting of tissue structures including the bottom membrane, epithelium

Table 1 | Variations in zooplankton composition in the effluent of different treatment processes (%; $n = 20$, $P < 0.05$)

	Process				
Zooplankton	Raw water	Sand filter	Ozone contact tank	GAC filter	Product water
Copepod	5.3 ± 0.3	70.2 ± 2.7	89.3 ± 3.7	95.0 ± 2.9	+
Cladocera	1.2 ± 0.1	2.0 ± 0.3	−	−	−
Rotifers	18.5 ± 1.1	1.5 ± 0.2	−	−	−
Protozoa	73.5 ± 2.6	20.3 ± 1.7	7.2 ± 1.1	2.3 ± 0.3	−
Others	1.5 ± 0.1	6.0 ± 0.4	3.5 ± 0.1	2.7 ± 0.1	−

Note: ' − ' indicates none detected; ' + ' indicates individual detection.

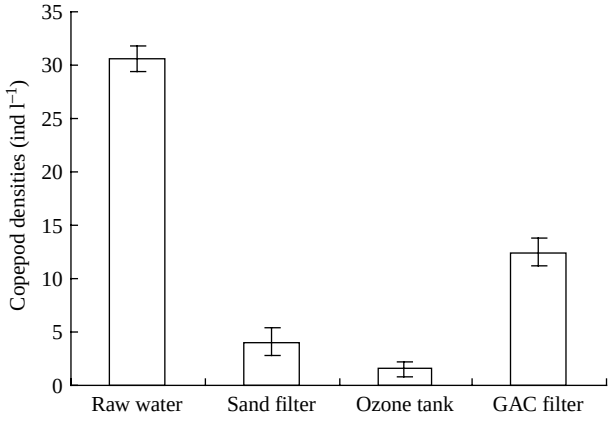

Figure 2 | Densities of Copepod in the effluent of different treatment processes. ($n = 20$, $P < 0.05$).

and calcific layer, etc. This structure provides Copepod with strong protection against inactivation by oxidation. Some studies have indicated that increased oxidant dosage and contact time are essential to thoroughly eradicate zooplankton (Liu *et al.* 2007). In addition, the inactivation rate of zooplankton would be influenced by organic matter in the water (Lin *et al.* 2004). In the above experiment, the concentration of the organic matter (represented as TOC) was about 4.5 mg l^{-1} in the ozone contact tank influent. Therefore, competition for ozone between oxidizing organic matter resulted in a decrease in the inactivation efficiency. The organic matter in water not only depletes the concentration, but also accelerates the decomposition of ozone, which results in a reduced exposure time for inactivation of the Copepod. As shown in Figure 3(b), the residual ozone was less than 0.1 mg l^{-1} after 20 min, regardless of the dosage applied. Due to the depletion of ozone, the exposure time was only about 20 min; therefore, the inactivation rates of Copepod were constant after 20 min for each ozone dosage experiment.

The contact time in the ozone contact tank of the waterworks evaluated in this study is 15 min; therefore, it seems that the most effective approach for complete removal of Copepod would be to increase the ozone dosage. However, the high bromide concentration in the raw water (average annual concentration ranging from 65 μg l^{-1} to 120 μg l^{-1}) prevents increasing the ozone dosage due to the production of bromate during ozone oxidation. Therefore, the inactivation rate and bromate production were investigated for different ozone dosage applied for 15 min contact time (25°C, TOC = 4.5 mg l^{-1}, pH = 6.89, bromide = 90 μg l^{-1}). As shown in Figure 4, when 90% inactivation was attained, the bromide concentration was already greater than the Standards for Drinking Water Quality stipulated in China (10 μg l^{-1}). These results indicate that high bromide concentration in raw water is the key factor preventing the complete inactivation of Copepod by ozone oxidation in the ozone contact tank.

Propagation of Copepod in the GAC filter

Copepod enters the GAC filter with the ozone contact tank effluent; therefore, it is possible for these organisms to propagate in the GAC filter. The water quality of the GAC filter effluent and the sand filter effluent are shown in Table 2. In the present study, similar growth conditions were created by artificial methods, which resulted in food becoming the key factor influencing the propagation of

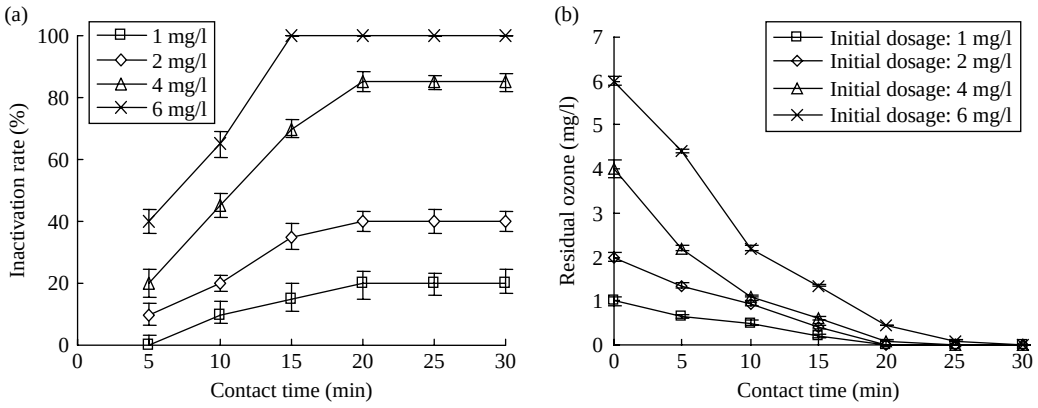

Figure 3 | Inactivation rate of Copepod (a) and residual ozone (b) at different ozone dosage and contact time ($n = 5$, $P < 0.05$).

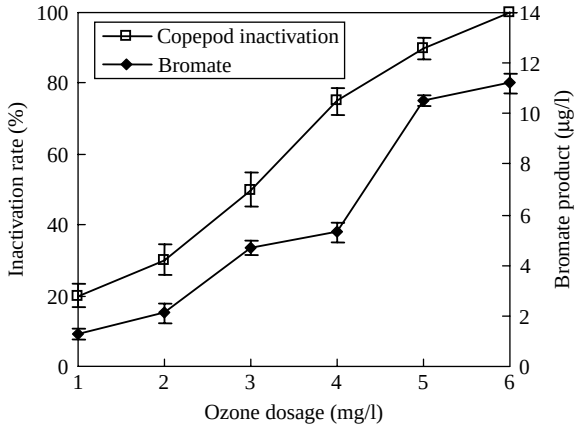

Figure 4 | Bromate production during inactivation of Copepod at different ozone dosage for 15 min contact time ($n = 5$, $P < 0.05$).

Table 2 | Comparison of water quality of the GAC filter and the sand filter ($n = 4$, $P < 0.05$)

	DO ($mg\,l^{-1}$)	Total bacteria ($cfu\,ml^{-1}$)	Algae ($ind\,l^{-1} \times 10^5$)	Organic debris ($ind\,l^{-1}$)
Sand filter	7.1 ± 1.1	290.0 ± 11.0	7.3 ± 0.8	53.0 ± 9.0
GAC filter	11.8 ± 2.1	734.0 ± 17.0	2.3 ± 0.7	712.0 ± 14.0

Copepod in cultures that contained different water samples (Table 3). As shown in Table 2, the proper environment for Copepod growth was provided by the GAC filter. Specifically, the GAC filter has (i) a high dissolved oxygen concentration (DO) due to the ozone oxidation; (ii) rich bacterial populations and high densities of algae and organic debris that provide Copepod with an abundance of food. Moreover, the adsorptive properties and porous surfaces of the carbon provide a protection for Copepod growth. As shown in Table 3, the clearance and feeding rates of Copepod under the simulated GAC filter environment were obviously higher than in the sand filter environment.

The proper feeding rate resulted in favorable growth of Copepod in the GAC filter (Figure 5). Additionally, the average period of Copepod growth (Figure 5(a)) decreased as the culture temperature increased, which corresponded with the increasing densities of Copepod in the raw water and GAC filter effluent during summer. As shown in Figure 5(b), the average period of Copepod growth was about eight days when the GAC filter water was 25°C, which was obviously less than that in sand filter water at the same temperature (21 days). As shown in Table 4, Copepod nauplii grew well when there was abundant food, which enabled it to attain a higher survival ratio in the GAC filter than in the sand filter.

Copepod observed in the present study belongs in Arthropoda of Crustacea, which are prone to inhabit water with high dissolved oxygen concentration, proper temperature and abundant food. Copepod primarily feeds on bacteria, organic debris, algae and protozoan or rotifers. However, it has been shown that the feeding rate of zooplankton primarily depends on the food abundance, and that it will decrease or stop when there is little food available (Perhar & Arhonditsis 2009). Therefore, more bacteria, algae and organic debris in the GAC filter are likely the main reason for the higher feeding rate of Copepod when compared to the sand filter. It is well known that zooplankton plays an important role in the food chain, feeding on bacteria, organic debris and algae, while being fed on by fish. However, in the GAC filter there are no fish, which exacerbates the excess propagation of Copepod in this environment.

The motility of Copepod enables it to easily penetrate the GAC filter and enter into the clear water reservoir. The change of Copepod density in GAC filter effluent was investigated and the experimental results are shown in Figure 6. The density of Copepod in the effluent fluctuated irregularly during a GAC filter cycle of 48 hours and the average value was $24\,ind\,l^{-1}$. Both nauplii and adult Copepod were found in the effluent, in which the

Table 3 | Effects of culture environment on the feeding rate of Copepod ($n = 4$, $P < 0.05$)

	10°C		20°C		30°C	
Artificial culture	***F***	G_c	***F***	G_c	***F***	G_c
Sand filter water	0.02 ± 0.003	2.5 ± 0.8	0.04 ± 0.005	4.2 ± 1.1	0.05 ± 0.004	4.8 ± 1.3
GAC filter water	0.06 ± 0.007	5.3 ± 1.3	0.13 ± 0.007	12.3 ± 2.8	0.15 ± 0.009	13.6 ± 2.8

F—Clearance rate ($ml\,ind^{-1}\,h^{-1}$); G_c—Feeding rate ($10^{-6}\,\mu gC\,ind^{-1}\,h^{-1}$).

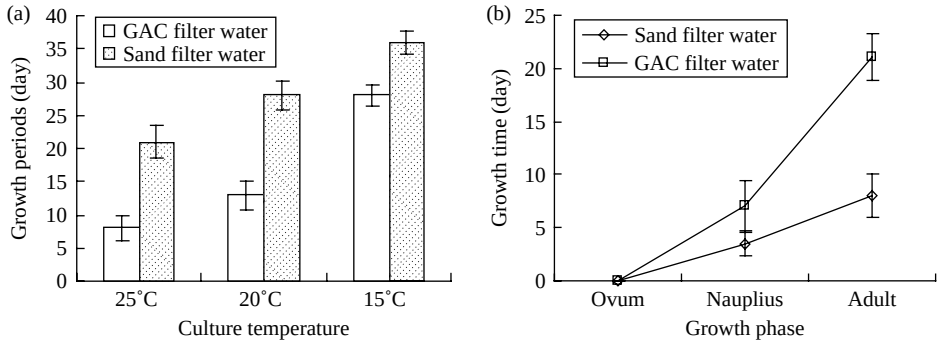

Figure 5 | Growth period of Copepod under different culture conditions ($n = 4$, $P < 0.05$).

percentage of adults was in the range of 45% ~ 80%. These findings indicate that there was no difference in the proportion of nauplii and adults in the population of Copepod. Taken together, these results indicate that the occurrence of Copepod in the GAC effluent is a result of vertical migration and the inability of filter media to trap the organisms. Adult Copepod has a strong capability of vertical migration, but is more easily trapped by the media, whereas the opposite is true for nauplii. However, the experimental results indicate that the self-migration accounts for the predominance during the penetration of both adult and nauplii in the GAC filter.

Table 4 | Average density of nauplii and adults grown in different culture media (ind l^{-1}) ($n = 4$, $P < 0.05$)

	Nauplii	Adult	Survival ratio (%)
Culture in GAC filter water	11 ± 2	9 ± 1	82.3 ± 8.4
Culture in sand filter water	7 ± 1	3 ± 1	43.0 ± 4.7

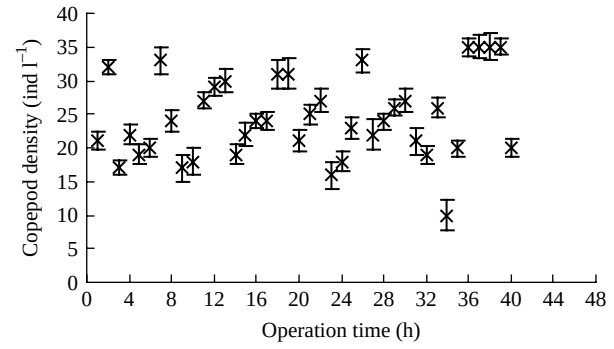

Figure 6 | Change in the density of Copepod in the GAC filter cycle ($n = 20$, $P < 0.05$).

Inactivation effect of Copepod by disinfection of chloramines

The following disinfection process is essential to eradicate Copepod in the effluent of the GAC filter for drinking water security. Different CT values (product of disinfectant concentration and contact time) were added to a given volume of distilled water. The experimental results of Copepod inactivation with chloramines and chlorine (at 25°C and pH 7) are shown in Figure 7. As shown in Figure 7(a), when the CT value was low, the inactivation rate of chlorine was better than that of chloramines. However, as the disinfectant concentration increased, there was less difference in the inactivation of Copepod by chloramines and chlorine. There was also little variation in the inactivation rate of chlorine when the contact time was greater than 30 min, whereas inactivation occurred for 40 min or more during chloramine disinfection. It also appears that Copepod was more sensitive to high disinfectant concentration and short contact time under the same CT value conditions. The tissue structure provides zooplankton with strong protection against disinfection; therefore, they may not be effectively inactivated unless the disinfectant destroys their surface tissue or penetrates directly into their body to oxidize inner proteins. Some studies of bacteria disinfection have indicated that there was a different disinfection mechanism for chlorine and chloramines (Norton *et al.* 2004). After dissolving in water, chloramines do not react with water molecules like chlorine, which enables chloramines to easily permeate Copepod tissue to destroy inner protein (Luh *et al.* 2008; Berry *et al.* 2009). In addition, the long exposure time

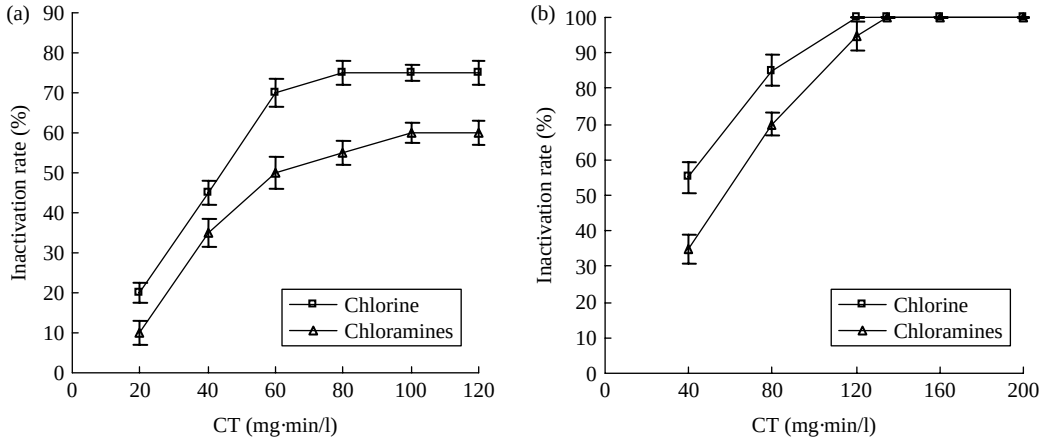

Figure 7 | Inactivation rate of Copepod at 25°C and pH 7 in response to different disinfectant concentration: (a) 2.0 mg l^{-1}, (b) 4.0 mg l^{-1}.

results in chloramines having a similar inactivation effect against Copepod under increasing disinfectant concentration and contact time.

Bench experiments were conducted to evaluate the inactivation rate at pH values ranging from 6 to 10 using a disinfectant concentration of 4 mg l^{-1}, which was selected based on the CT values in above neutral conditions for the complete inactivation of Copepod. As shown in Figure 8, pH values in the range from 6 to 10 did not influence the inactivation efficiency of chloramines. Different products are created by chemical reaction between chlorine and ammonia when the pH differs, with monochloramine being the predominant product formed at pH values from 6 to 10. However, the addition of chlorine to water results in the formation of HOCl and OCl^-. HOCl plays the main role in the bactericidal function of chlorine. Despite this, the predominant disinfectant species change from HOCl at lower pH values (such as pH 6 and 8) to OCl^- at higher pH values (such as pH 10), which results in a decrease of inactivation rate when the pH value increases.

Various experiments were conducted to investigate the relationship between the inactivation rate and organic matter concentration. The concentration of chloramines was 4 mg l^{-1}in this study.

The experimental results shown in Figure 9 indicate that the organic matter had a significant influence on the inactivation of Copepod by chlorine. Organic matter is composed of reductive substances; therefore, it may react with chlorine, resulting in a decreased concentration being available to inactivate Copepod and a concordant decrease in chlorine inactivation efficiency. However, chloramines have a rather low capacity for oxidizing organic matter and its inactivation rate is thus less influenced by organic matter concentration.

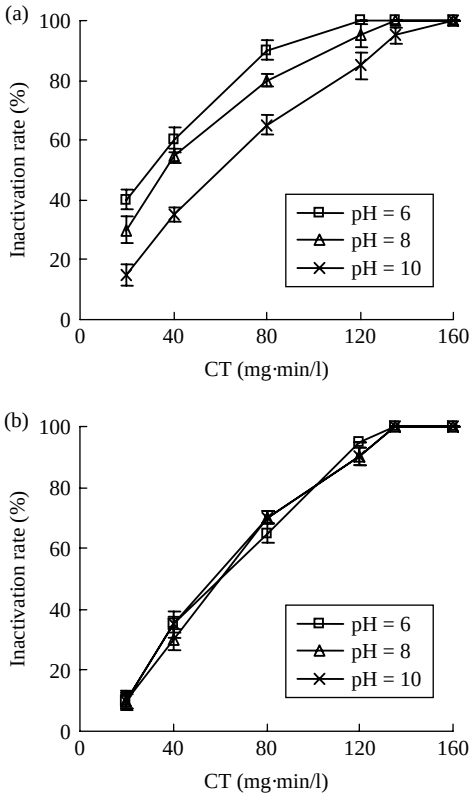

Figure 8 | Effect of pH on inactivation rate of Copepod at 25°C in distilled water: (a) chlorine (b) chloramines ($n = 5$, $P < 0.05$).

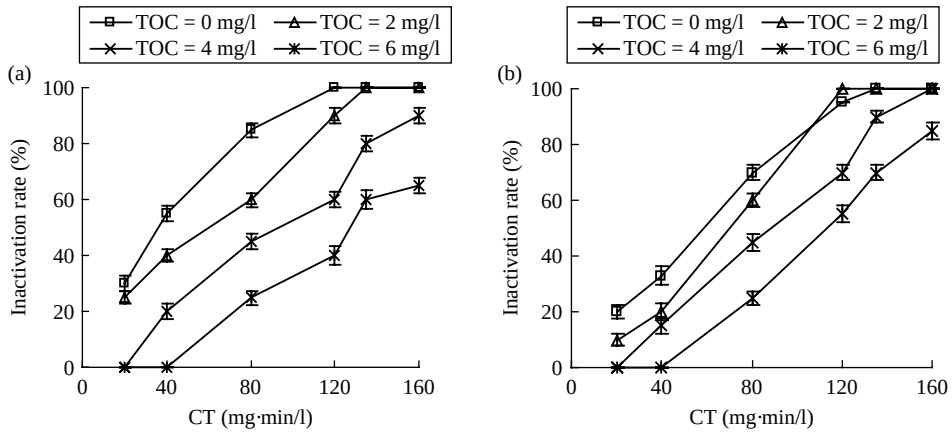

Figure 9 | Effect of organic matter on inactivation rate of Copepod at 25°C and pH 7 (a) chlorine, (b) chloramines ($n = 5$, $P < 0.05$).

Disinfection effect of chloramines in the GAC filter effluent

To better understand the behavior of the disinfection process, the effluent from the GAC filter was used in laboratory experiments (Figure 10). The chloramines more effectively inactivated Copepod with high CT values ($>80\,mg\,min\,l^{-1}$) when compared with chlorine. In contrast to the distilled water experiment, many organic micropollutants as well as bacteria exist in the GAC filter effluent. These substances result in the competition for chlorine and a concordant decrease in Copepod inactivation rate when the contact time is increased. However, chloramines have a low oxidation capacity and are less influenced by organic matter than chlorine; therefore, no decrease in the inactivation rate occurs.

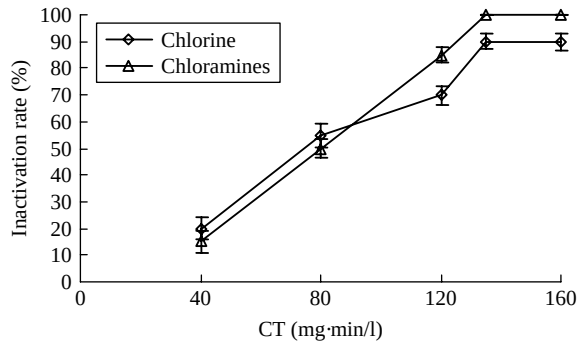

Figure 10 | Inactivation rate of Copepod by chlorine and chloramines in GAC filter effluent ($n = 5$, $P < 0.05$).

To estimate the treated water quality, the disinfection byproducts of THMs were investigated at a CT value of $160\,mg\,min\,l^{-1}$ (disinfectant concentration of $4\,mg\,l^{-1}$). As shown in Table 5, only one third of the total amount of THMs was produced in response to treatment with chloramines when compared with the amount produced by treatment with chlorine. Additionally, more brominated trihalomethanes, such as $CHBr_3$, were produced in water samples that were treated with chlorine. During the oxidization of organic matter by chlorine, electrophilic substitution results in the formation of more mutagenic halogenated hydrocarbons such as trichloromethane. However, for chloramines, there is less electrophilic substitution during disinfection. Hypobromous acid (HOBr), which has a high chemical reactivity and more easily reacts with organic matter than hypochlorous acid (HOCl), is also created by the reaction between chlorine and bromide (HOCl) (Butler *et al.* 2005; Do *et al.* 2008).

Table 5 | Varieties of THMs product during different disinfection process ($\mu g\,l^{-1}$) ($n = 5$, $P < 0.05$)

	$CHCl_3$	$CHCl_2Br$	$CHClBr_2$	$CHBr_3$	THMs
Chlorine	18.8 ± 1.1	7.7 ± 0.8	11.5 ± 1.2	15.3 ± 0.9	53.3 ± 3.1
Chloramines	8.6 ± 0.9	6.4 ± 0.5	3.6 ± 0.4	–	18.6 ± 1.3

Note: TOC = $3.51\,mg\,l^{-1}$, $T = 25$°C, $[Br^-] = 70\,\mu g\,l^{-1}$; '–'indicates none detected.

CONCLUSION

The increasing Copepod density in the eutrophic water source of the treatment facility evaluated in this study and poor removal efficiency by the traditional treatment process resulted in Copepod penetration from the sand filter into the ozone contact tank. The high bromide concentration in the raw water was the key factor that prevented the inactivation of Copepod by increasing the ozone dosage. Surviving Copepod then entered the GAC filter via the effluent of the ozone contact tank. Because the concentrations of bacteria, algae and organic debris in the GAC filter were high, the clearance and feeding rates of the Copepod were obviously higher than in the sand filter environment, which provided Copepod an opportunity for propagation. The inactivation of Copepod occurred for 40 min or more during chloramine disinfection, resulting in chloramines having a similar inactivation effect to that of chlorine under increasing chloramine concentration and contact time. In addition, the Copepod disinfection of chloramines was less influenced by extraneous factors such as pH value and the presence of organic matter.

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