Removal efficiency of invertebrates in the filtrate of biologically activated carbon filter with sand bed

Wen-Chao Yin, Jin-Song Zhang, Li-Jun Liu, Yan Zhao, Tuo Li and Chao Lin

ABSTRACT

In view of the aesthetic problem and potential threat to safe drinking water caused by invertebrates, a series of different depth sand beds were located under granular activated carbon (GAC) media in five pilot-GAC filtration (GAC_F) columns to restrict invertebrates' access into the distribution system. During the study period May 2010 to March 2011, seven groups of invertebrates (rotifers and crustaceans as the predominant species) were detected in the filtrates of the five GAC_F columns. The experimental results indicated that invertebrates could be removed effectively with the added sand beds compared with the sand bed-free GAC_F column. The mean abundances of invertebrates decreased significantly with the increase in the depth of sand beds, while there were different removal ratios between rotifers (29.8–46.6%) and larger invertebrates (size >200 μm) (41.7–85.5%). Sand sizes had a greater impact on rotifer removal than on larger invertebrates. Also, increasing removal ratios of particle matter were detected with the sand beds added. Further data analysis showed that there was significant correlation between the mean values of particle counts and abundances of invertebrates in the filtrates. The mixed-layer phenomenon between the GAC and the sand media could be controlled effectively under the optimized backwashing procedures.

Key words | drinking water, GAC filtration, invertebrates, sand beds

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INTRODUCTION

In temperate countries, the presence of invertebrates in drinking water has largely been considered by water suppliers because the larger invertebrates (Cyclops, chironomid larvae, and Oligochaeta, among others) may be visible to the consumers and the decay and feces of invertebrates may bring taste and odor problems (WHO 2004). In addition, further study has shown that the isolated bacteria from the invertebrates recovered from distribution mains in South Africa included several bacterial genera and species that were either pathogenic or opportunistic pathogens of humans (Wolmarans *et al.* 2005). In recent years, increasing scientific attention has been paid to invertebrates due to their important role as vectors and protectors of waterborne pathogens (Locas *et al.* 2007; Bichai *et al.* 2008, 2009; Loret *et al.* 2008) and in the transport and fate of protozoan (oo)cysts (Bichai *et al.* 2010), which are becoming more accurately described and highlighted as a potential threat to water safety.

In view of the poor removal efficiency of organic matter from source water in the most conventional water treatment systems, granular activated carbon (GAC) as the filtration media has been employed to improve the quality of drinking water due to its irregular creviced and porous particle shape. With the increase of running time of GAC filters, the rough porous surfaces of the GAC filtration (GAC_F) media becomes increasingly covered with biofilm to a steady maximum which depends on the dynamics of back washing and substrate supply (Scholz & Martin 1997; Takeuchi *et al.* 1997). At this stage, the GAC process is known as the biologically activated carbon (BAC) process, which overcomes several limitations of the GAC process and is considered an advanced water treatment process (Simpson 2008). However, the natural or artificially enhanced biofilm and

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particulate debris which are intercepted by the GAC_F media are both a good food resource for invertebrates. So it can be understood why GAC filters are more easily colonized by invertebrates and have a higher abundance in the filtrate compared with other water treatment units (Schreiber *et al.* 1997; Castaldelli *et al.* 2005; Weeks *et al.* 2007; Li *et al.* 2010).

It is well known that the GAC/BAC filters are in general the last stage in water treatment stage. After the bio-filtration process, a final disinfection is necessary to ensure the microbial security in the distribution system (Pernitsky *et al.* 1995). It has been widely proved that free-living or attached bacteria could be disinfected efficiently by conventional disinfection methods, however further studies have suggested that the bacteria or pathogens ingested and harbored by invertebrates could survive disinfection. Chang *et al.* (1960) and Smerda *et al.* (1971) successively confirmed that nematodes in the municipal supplies could protect human enteric pathogens and salmonellae from chlorination. Levy *et al.* (1984) fed amphipods with suspensions of *Escherichia coli* and *Enterobacter cloacae* and exposed them to chlorination (1 mg/L for 60 min), homogenized the animals and determined the count of viable bacteria. Viability of the bacteria in or on the amphipods was reduced to about 2% (*E. coli*) and 15% (*E. cloacae*). In contrast, bacteria that had not been in the presence of the amphipods were reduced to about 1% in 1 min at the same concentration of chlorine. A recent study proved that internalization of *E. coli* bacteria and *Bacillus subtilis* spores by *Caenorhabditis elegans* nematodes was shown to offer significant protection against UV disinfection at the typical fluence of 40 mJ/cm^2 applied in the water treatment industry (Bichai *et al.* 2009).

In view of aesthetic problems and the potential threat to safe drinking water caused by invertebrates as mentioned above, it is important to prevent the invertebrates' penetration through the BAC filters and control them at levels as low as technically and economically feasible; however, few studies have focused on the operation optimization of GAC filters for invertebrates' penetration control. It is long-established that an optimized backwashing procedure (9 m/h pressure air for 15 min; 16.8 m/h drinking water for 10 min; 33.2 m/h drinking water for 20 min) can reduce this biomass output into the distribution system; however, the abundance of invertebrates in the filtrate was still at a high level (1,720 ind./m^3) after the above backwashing procedure conducted when the invertebrates breed excessively in the GAC filters (Schreiber *et al.* 1997). In addition, another study suggested that routine backwashing altered the spatial distribution of snails, but not their overall abundance. In small-scale glass columns 40–50% of the smallest (0.3–0.6 mm shell height) juvenile snails were removed by a GAC backwash bed expansion of 30–40%; however, bed expansions of greater than 20% were not possible in the GAC adsorbers (Weeks *et al.* 2007). It can be expected that the removal capacity of invertebrates is limited by a simple optimization of the backwashing procedures. In this case, it is necessary to develop an effective method to control the penetration of invertebrates through GAC filters. Some researchers pointed out that the sand process could effectively remove the invertebrates from source water (Adam *et al.* 1998; Liu 2010). Adam *et al.* (1998) pointed out that newer rapid sand gravity filters are able to maintain invertebrate levels within acceptable limits. Liu (2010) indicated that after the rapid sand process the invertebrates from the raw water could be removed by 98.1 and 99.9% in two waterworks in South China, thereby in filtrate of the sand filters the monitored abundance of crustaceans was not higher than 40 ind./m^3. In view of previous studies, we wanted to determine whether a sand bed located under GAC_F media could prevent the penetration of invertebrates. Consequently, this study aims to confirm the removal efficiency of invertebrates in the filtrate of BAC filters with sand beds, with specific reference to the sand bed depth and sand particle sizes.

MATERIAL AND METHODS

GAC filtration systems

As shown in Figure 1, a pilot system with five parallel GAC_F columns was installed in an ozonation-biologically activated carbon (O_3-BAC) waterworks located in Shenzhen city, South China, which is supplied by the Dongxiang River water after storage in the Shenzhen Reservoir. Being a subtropical zone, the average temperature of the source water is above 23 °C, which provides an ideal physical environment

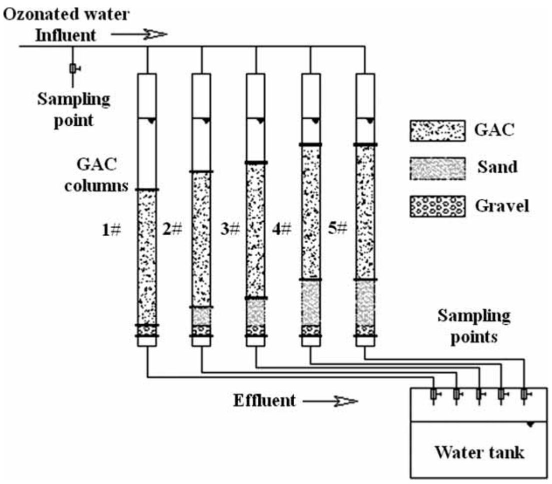

Figure 1 | Supply diagram of GAC filtration columns.

for the growth and reproduction of invertebrates in the fixed bed GAC filters. The composition and physical characteristics of the five columns are summarized in Table 1. All columns were similar except the depth of the sand bed and the sizes of the used sand.

The GAC_F columns ($8.0\ m\ h^{-1}$ and contact time of 11.25 min; no backwashing) were operated from May 2010 to March 2011. The GAC_F columns received ozonated water from the treatment plant. The quality of ozonated water is summarized in Table 2.

Sampling and analysis

The invertebrate samples were collected with a commercially available plankton net of 35 μm mesh size for 4 h and 942 L of GAC filtrate water. The samples were collected twice per week from May 2010 and October 2010 and weekly from October 2010 to March 2011. After each sampling, the net was rinsed off with distilled water to avoid contamination among sampling. Samples were fixed with 3% formalin into 50 mL within the same day. Invertebrates were observed and counted with an inverse microscope (BX-51, Olympus, Japan) at 100× and photographed with the same microscope and an image system software (Mshot MD 20). The invertebrates' body length was measured with software (SPOT advanced) using an ocular micrometer.

Samples of influent and filtrate of the GAC columns were taken for analysis of turbidity, particle counts, chemical oxygen demand ($KMnO_4$) (COD_{Mn}), temperature (T), dissolved oxygen (DO) and pH at the same time as the invertebrates samples were taken. Turbidity and pH were

Table 1 | Physical characteristics of GAC filtration columns

Physical property	Column 1#	Column 2#	Column 3#	Column 4#	Column 5#
Diameter of carbon pellet (mm)	0.9–1.1				
Shape of carbon pellet	Irregular				
Diameter of column (m)	0.2				
Height of column (m)	2.6 (Transparent plexiglass)				
Height of carbon bed (m)	1.5				
Height of sand bed (mm)	0	200	300	500	500
Ganister sand grading (mm)	–	0.6–1.0	0.6–1.0	0.6–1.0	0.8–1.2
Height of gravel layer (mm)	100				
Gravel layer grading (mm)	3–12				

Table 2 | Quality of the influent of GAC filtration columns

Parameters	Water temperature (°C)	pH	Turbidity (NTU)	Dissolved oxygen (mg/L)	COD_{Mn} (mg O_2/L)	Residual chlorine (mg/L)
Range	13.9–31.3	7.41–9.56	0.11–0.79	4.7–11.0	0.72–2.31	0.07–0.80
Average	25.3	8.48	0.20	7.64	1.27	0.27

determined by a portable turbidimeter (2100P, HACH, USA) and portable pH meter (Orion 3-Star Plus, Thermo Scientific, USA). Particle counts (size >2 μm) were tested and calculated with a particle counter (L V Versacount particle, IBR, USA). COD_{Mn} was measured in accordance with the 'fourth edition of water and wastewater quality monitoring, China' (Chinese NEPA 2003). Temperature and DO were measured with the help of a handheld dissolved oxygen meter (550A, YSI, USA).

Statistical analysis

Significant statistic difference and correlation analysis were carried out using SPSS 13.0. 2-Related samples tests were conducted to examine the significant difference of the particle counts between influent and filtrate of all five GAC_F columns, while Pearson correlation tests were conducted to examine the correlation between the mean particle counts and mean abundances of invertebrates in the filtrates of five GAC_F columns. The box-and-whisker plots and other tendency charts were carried out using Origin 8.0.

RESULTS

Invertebrate species composition and abundance comparison

During the study period, seven groups of organisms including rotifers, cladocerans, copepod adults (two species), copepod nauplii, nematodes, chironomid larvae and oligochaetes were detected in filtrates of all five GAC_F columns. Table 3 summarizes the data of mean values with the standard deviations, range (between minimum and maximum) and values of abundances for all groups of invertebrates in the different filtrates. In all samples, rotifers were the dominating taxonomic group. In the initial stages of GAC filters operation, most of the detected rotifers belonged to *Philodina* sp.; however, in most of the running time, *Lepadella* sp. (60%) dominated the rotifer fauna followed by *Monostyla* sp. (12%), *Colurella* sp. (3%), and other species not identified made up the rest. In terms of numbers, copepods were in - general the second important group, which included copepod adults (represented by *Nitocra pietschmanmi* and

Table 3 | Mean and range values of abundances of invertebrates in the filtrates of GAC filtration columns

N = 48

Organism group	Column 1#		Column 2#		Column 3#		Column 4#		Column 5#	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Rotifers	2,160 ± 4,047	0–17,030	1,517 ± 2,700	0–13,000	1,242 ± 2,364	0–11,080	1,153 ± 2,041	0–9,780	1,531 ± 3,584	0–16,760
Copepod nauplii	72 ± 201	0–1000	58 ± 242	0–1677	27 ± 47	0–173	16 ± 25	0–124	18 ± 48	0–315
Mesocyclops thermocyclopoides	50 ± 138	0–887	49 ± 134	0–809	14 ± 18	1–60	13 ± 16	0–112	15 ± 23	0–116
Nitocra pietschmanmi	82 ± 512	0–3,500	13 ± 39	0–235	9 ± 29	0–198	4 ± 7	0–47	10 ± 35	0–231
Alona diaphana	1.4 ± 1.9	0–11	1.1 ± 2.0	0–12	1.1 ± 1.5	0–6	0.7 ± 1.1	0–5	0.8 ± 1.5	0–6
Chironomid larvae	2.7 ± 4.5	0–20	2.9 ± 3.8	0–16	2.1 ± 2.9	0–15	1.0 ± 1.6	0–9	2.7 ± 6.2	0–35
Nematodes	0.23 ± 0.69	0–4	0.21 ± 0.74	0–4	0.21 ± 0.68	0–4	0.19 ± 0.57	0–3	0.04 ± 0.20	0–1
Oligochaetes	0.15 ± 0.62	0–7	0.31 ± 1.31	0–8	0.13 ± 0.39	0–2	0.13 ± 0.49	0–3	0.08 ± 0.35	0–2
All organisms	2,369 ± 4,180	5–17,548	1,640 ± 2,713	6–13,078	1,296 ± 2,387	1–11,296	1,188 ± 2,071	1–9,953	1,578 ± 3,592	2–16,798

N: number of sampling; mean: mean ± standard deviation values of abundance (ind./m^3); range: minimum–maximum values of abundance (ind./m^3).

Mesocyclops thermocyclopoides) and copepod nauplii. Cladocera and oligochaeta were represented by *Alona diaphana*, *Nais bretscheri* Michaelsen respectively with nematodes and chironomid larvae not identified further.

As shown in Table 3, the mean and maximum abundances of rotifers, copepod adults and nauplii decreased significantly with the sand beds added. Compared with the sand bed-free GAC$_F$ column, in the filtrates the removal ratios of mean abundance had a gradual increase with the increase in the depth of sand beds, which ranged from 29.8 to 46.6% for rotifers and from 41.7 to 85.5% for other larger invertebrates tested. The maximum removal ratios of the mean abundance occurred at column 4# with 500 mm depth of sand bed (sand size 0.6–1.0 mm), which were 46.6% for rotifers, 77.8% for copepod nauplii, 74.0% for *M. thermocyclopoides* and 95.1% for *N. pietschmanmi*. Compared with column 4#, there were similar removal ratios of copepod adults and nauplii but a relatively lower removal ratio of rotifers for column 5# with equal 500 mm depth of sand bed (larger sand size 0.8–1.2 mm). Because of originally low abundances, there was no significant variation for mean abundances of cladocerans, chironomid larvae, nematodes and oligochaetes in the filtrates among the GAC$_F$ columns.

As shown in Figure 2, box and whisker plots of abundances of rotifers and overall invertebrates (size >200 μm) had been drawn, which indicated that invertebrates could be removed effectively with the sand beds added more spontaneously. As shown in Figure 2(a), for rotifers the maximum values, mean values and standard deviations of abundances decreased gradually with the increase in the depth of the sand beds (columns 2#–4#), which had smaller sand size (0.6–1.0 mm), whereas sand beds with larger sand size (0.8–1.2 mm) (column 5#) proved to have little effect in preventing the penetration of rotifers. Compared with the rotifers, for the larger invertebrates (size >200 μm) the maximum values of abundances showed a more significant decrease, and smaller mean values of abundances with a significant decrease in standard deviations were obtained by increasing the depth of the sand beds. Regarding the acceptable abundance limit of invertebrates in drinking water, 200 ind./m^3 was suggested as the maximum permissible limit by Rand Water in South Africa (Adam *et al.* 1998) and 50 ind./m^3 was used as the recommended limit in Shenzhen city, South China. It was therefore seen that the abundance of invertebrates (size >200 μm) in the filtrate could also meet the above limits with a larger size of sand bed (column 5#). However, considering its negligible effect for removing rotifers, the smaller size of sand bed at a height of 500 mm was recommended in our current research.

The measured results had shown that body lengths of invertebrates were quite different according to different species of organisms in the filtrates of GAC$_F$ columns. Lengths of rotifers were in general near 110 ± 10 μm, copepod nauplii (200 ± 20 μm), copepod adults: *N. pietschmanmi* (520 ± 30 μm) and *M. thermocyclopoides* (920 ± 50 μm), cladocera: *A. diaphana* (380 ± 20 μm), oligochaeta: *N. bretscheri* Michaelsen (1,200 ± 100 μm) and chironomid larvae (2,000 ± 100 μm). The theoretical calculated gap sizes of sand beds were 92.8–154.7 and 123.8–185.6 μm for sand size of 0.6–1.0 and 0.8–1.2 μm, respectively. It could

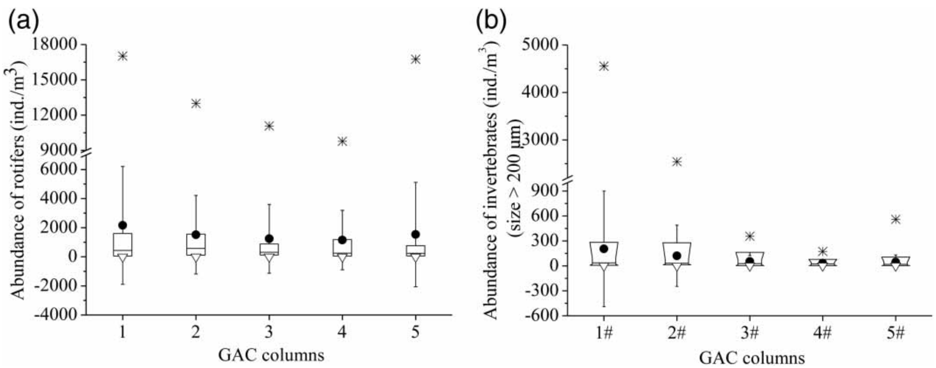

Figure 2 | Box and whisker plots of abundances of rotifers (Figure 1(a), box: 25%, 75%) and overall invertebrates (size >200 μm) (Figure 1(b), box: 10%, 25%, 75%, 90%) in the filtrate of GAC filtration columns 1#–5#; * represents the maximum values; ▽ represents the minimum values; • represents the mean values; whiskers represent the standard deviations.

be explained that there was significant difference in the removal effect between rotifers and other larger organisms by comparing the body lengths of invertebrates and the gap sizes of sand beds.

Time-related abundance variations of different groups of invertebrates

Figure 3 shows the abundance variations of six taxonomic groups of invertebrates with the increase of running time during the study period, which include rotifers, cladocerans, copepod adults (two species), copepod nauplii and chironomid larvae. In view of originally low abundances during the whole study period, the abundance changes of nematodes and oligochaetes were not plotted here.

As shown in Figure 3, firstly for rotifers, there were significant differences in the peak numbers, peak values and the maximum peak-arrival time among the four columns. There were five peaks for column 1#, while four peaks for both column 2# and column 3#, and only three peaks for

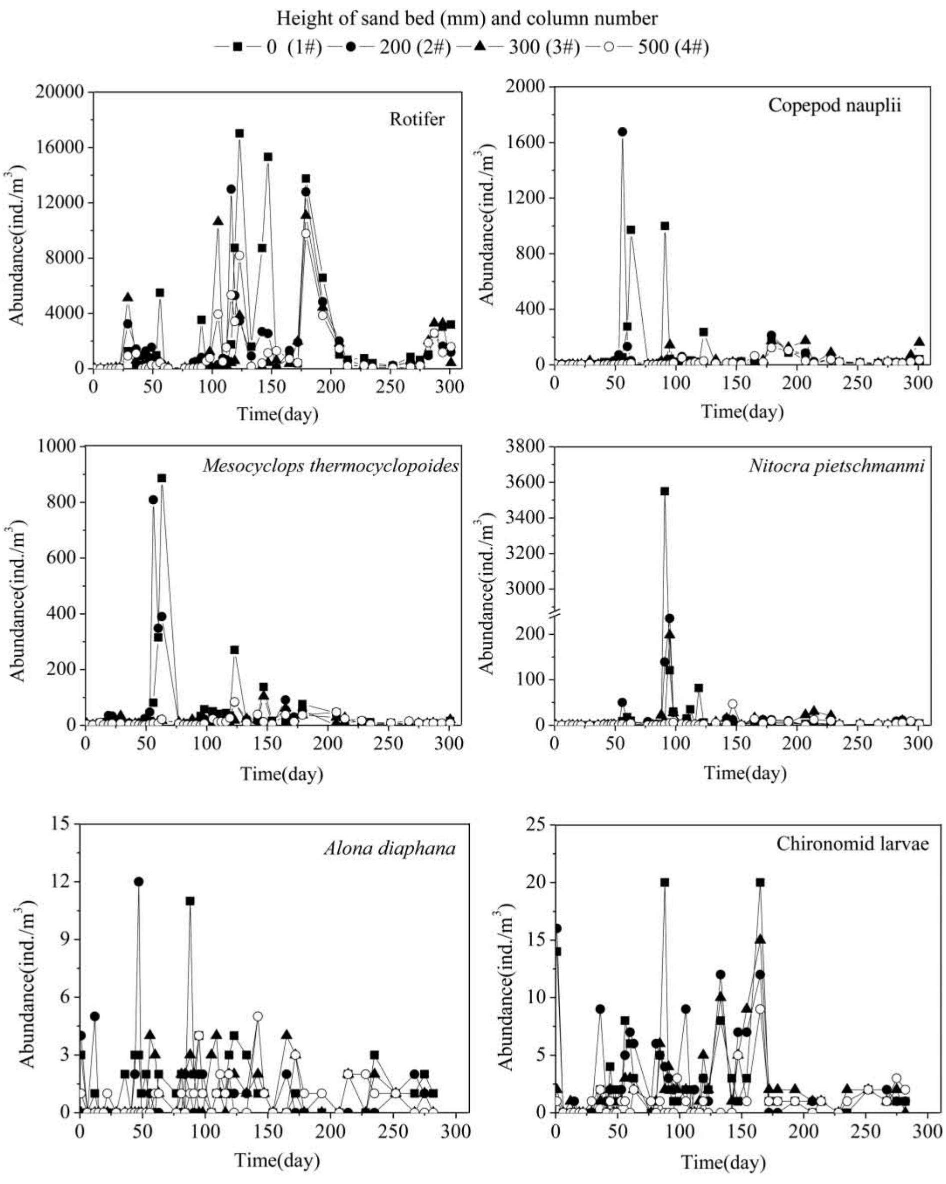

Figure 3 | Time-related abundance changes of six groups of invertebrate in the filtrates of GAC filtration column 1#-column 4# during the study period.

column 4#. The maximum peak values were 17,030 ind./m^3 (column 1#) > 13,000 ind./m^3 (column 2#) > 11,080 ind./m^3 (column 3#), and 9,780 ind./m^3 (column 4#). The maximum peak-arrival time was day 110–130 for column 1# and column 2#; however, this was 2 months later for column 3# and column 4# (day 180). Second, for copepod nauplii, there were three significant peaks for column 1# (day 60, 120 and 150) and one peak for column 2# (day 60), which was the topmost abundance value of 1,677 ind./m^3 in the filtrates of all four columns. Compared with columns 1# and 2#, there were much lower peak values for columns 3# (272 ind./m^3) and 4# (184 ind./m^3), both at day 180. For copepod adult *M. thermocyclopoides*, there was no significant abundance peak in filtrate of columns 3# and 4# (<100 ind./m^3), whereas the maximum peak values occurred at columns 1# and 2# at day 60 (both >800 ind./m^3). For another copepod adult, *N. pietschmanmi*, at day 90 there were peaks for columns 1#–3#; however, the peak values decreased with the increasing depths of sand beds. For column 4#, the abundance was continuously at a low level without any peak occurring during the whole study period. Although the abundances of *A. diaphana* and chironomid larvae were much lower than rotifers and copepods, there were obvious peak abundances occurring at columns 1# and 2#, whereas smooth fluctuations occurred at columns 3# and 4#.

Comparison of particle counts and COD_{Mn} in the filtrates

The mean and range values of particle counts and COD_{Mn} in the influent and the filtrates of GAC_F columns 1#–5# are summarized in Table 4. Table 5 summarizes the analysis results of significant statistical differences of particle counts (size >2 μm) in the influent water and the filtrates of all columns through Wilcoxon Signed Ranks Test, which is a non-parametric statistical hypothesis test used when comparing two related samples or matched samples. There were significant differences of particle counts between influent and the filtrates of columns 3#–5# ($p < 0.05$), whereas no significant difference was found between the influent and the filtrates of columns 1#–2#. Specially, the particle counts decreased significantly for column 4# compared with column 3# ($p = 0.000$) and column 5# ($p = 0.012$).

Table 4 | Mean and range values of particle counts and COD_{Mn} in the influent and the filtrates of GAC filtration columns 1#–5#[a]

Parameters		Influent	Filtrate 1#	Filtrate 2#	Filtrate 3#	Filtrate 4#	Filtrate 5#
Particle counts (size >2 μm)(ind./mL)	Mean	159 ± 265	101 ± 44	92 ± 36	75 ± 35	58 ± 27	76 ± 46
	Range	39–1,462	44–281	26–182	26–164	14–148	32–278
COD_{Mn} (mg/L)	Mean	1.27 ± 0.42	0.90 ± 0.34	0.92 ± 0.34	0.93 ± 0.35	0.91 ± 0.36	0.93 ± 0.35
	Range	0.72–2.31	0.57–1.97	0.54–1.96	0.48–1.88	0.31–1.88	0.54–2.14

[a]Number of sampling, $n = 48$.

Table 5 | Significant statistical differences of total particle counts >2 μm/mL in filtrate of GAC filtration columns[a]

2-Related samples tests (p)	Influent	Filtrate 1#	Filtrate 2#	Filtrate 3#	Filtrate 4#	Filtrate 5#
Influent	–					
Filtrate 1#	0.627	–				
Filtrate 2#	0.329	0.510	–			
Filtrate 3#	0.005	0.001	0.018[b]	–		
Filtrate 4#	0.000[c]	0.000[c]	0.000[c]	0.000	–	
Filtrate 5#	0.042[b]	0.001[c]	0.044[b]	0.885	0.012[b]	–

[a]Number of sampling, $n = 30$.
[b]Significance at $p < 0.05$.
[c]Significance at $p < 0.01$.

Figure 4(a) shows the counts distribution of more than 2 μm size of particles in the influent and the filtrates of all GAC columns. In the influent, the mean particle counts reached 159 ind./mL with a large standard deviation (SD = 265). In the filtrates of GAC_F columns, the mean values, maximum values and standard deviations of particle counts decreased significantly with the increasing depths of sand beds. There was a maximum decline of mean particle counts with a minimum standard deviation in the filtrate of column 4#. Compared with column 4#, although there was an equal depth of sand bed in column 5#, the removal effect of particle counts was unsatisfactory, which was considerably above the recommended limit (50 ind./ml) (Hargesheimer *et al.* 1998) and with a larger SD (46). Figure 4(b) shows the COD_{Mn} in the filtrate of all columns. The results showed no significant difference between columns with different depths of sand beds added. Compared with the influent, the removal ratios of COD_{Mn} ranged from 27.0 to 29.1% for all five GAC columns. Although the particle counts cannot directly represent the abundances of invertebrates in the filtrates in view of the relatively lower sampling volume for particle counting (300 mL), further data analysis by a Pearson correlation test showed that there was significant correlation between the mean values of particle counts (size >2 μm) and the mean abundances of invertebrates in the filtrates of five GAC filtration columns (Rotifers: $r_S = 0.883$, $p = 0.047 < 0.05$; Larger invertebrates: $r_S = 0.914$, $p = 0.03 < 0.05$).

Effect of backwashing on sand bed

Figure 5 shows the bed structure variety of GAC_F columns with the sand beds added. After 50 m/h air backwashing for 2 min it was seen that some sand grains penetrated upwards to the GAC media layer for all three columns (2#–4#). The mixed layer of the sand media and GAC media reached up to 200 mm. The interface of the carbon layer and sand layer became bumpy. As a result, the sand was washed into the above activated carbon media layer in the local area; however, there were no GAC particles involved into the lower sand layer. After air backwashing, 10 m/h of drinking water backwashing for 2 min was conducted. Although complete fluidization of the sand beds could not be obtained, the sand grains which were backwashed into the upper GAC layer by previous air backwashing were significant down to 100 mm from the interfaces. When the velocity of backwashing water increased to 20 m/h, there was an incomplete fluidization of the sand bed and GAC layer. As a result of hydraulic classification, a clear interface between the GAC layer and the sand layer improved before the air backwashing at 2.5 min and the sand grains were all reduced to less than 50 mm from the interface. It could be seen that complete fluidization occurred at the interface between the carbon layer and the sand layer, and also the inside sand layer when the flow velocity of backwashing water continued to increase to 30 m/h; however it was unfortunate that some carbon particles were drawn into the sand layer at 30 m/h due to the stronger hydraulic action.

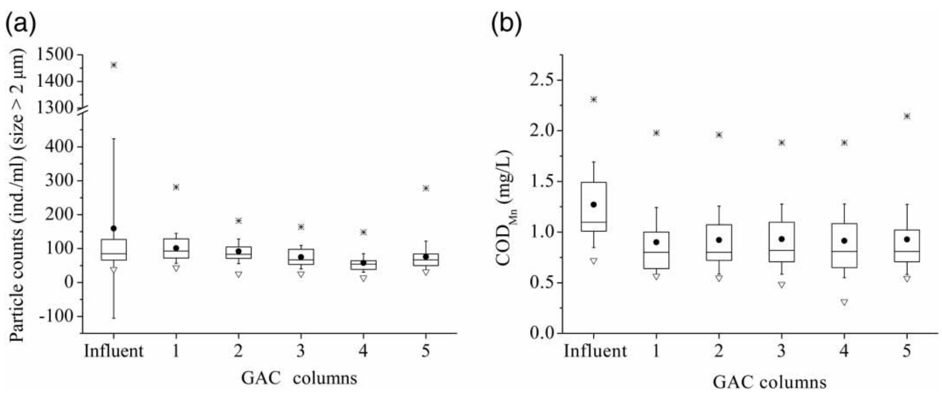

Figure 4 | Box and whisker plots of particle counts ((a), box: 25%, 75%) and COD_{Mn} ((b), box: 25%, 75%) in the influent and the filtrates of GAC filtration columns 1#–5#; ⋆ represents the maximum values; ▽ represents the minimum values; • represents the mean values; whiskers represent the standard deviations.

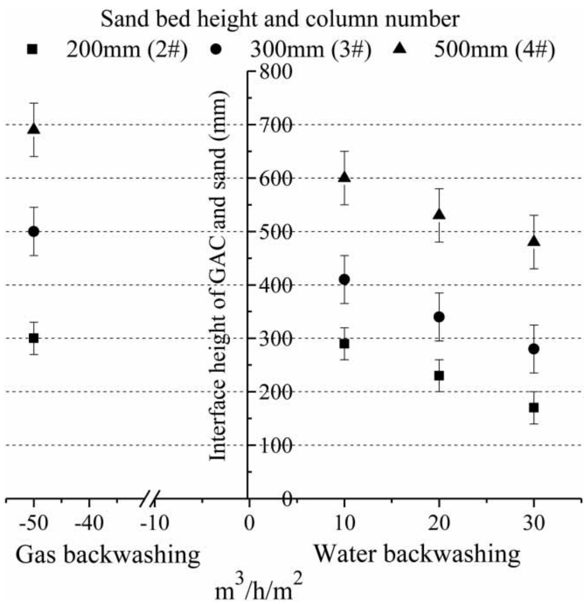

Figure 5 | Influence of backwashing (gas or water) on the bed structure of GAC filtration columns with the sand beds added (error bars show the range).

DISCUSSION

Invertebrate abundance and species composition

Since 1997, there have been several systematic surveys reported on the colonization of invertebrates in BAC filters and their occurrence in the filtrate (Schreiber *et al.* 1997; Bichai *et al.* 2009; Li *et al.* 2010). Although it was indicated that the mean or peak abundances were different in the investigations, rotifers were the dominating taxonomic groups without exception. Most of the rotifers belonged to *Colurella* spp., *Lepadella* spp., *Bdelloidea* spp., and *Monostyla* spp. A similar conclusion was attained in our current study. Nematodes were found as the second important group, generally in Germany (Schreiber *et al.* 1997) and the Netherlands (Bichai *et al.* 2009), while their second position was replaced by copepod nauplii and adults in China by Li *et al.* (2010) and our current study. In this study, the maximum peak abundance of rotifers was 17,030 ind./m^3 (column 1# as shown in Figure 3), which was twice the abundance (Li *et al.* 2010). The peak-arrival times of important groups ranged from 30 to 120 days, which were much earlier than the results of Li *et al.* (2010). In view of the similarity in operation parameters and structure of the BAC filters bed, it is speculated that the difference is due to the higher sampling frequency and higher-temperature starting environment of GAC systems in our study. Although the peak abundances appeared at different operation times, the peak-arrival time was exactly distributed in July to September, the highest monthly temperatures in Shenzhen city. These phenomena can be explained by some researchers who indicated that different seasonal temperatures affected the metabolic rate of aquatic invertebrates, which were shown in changes in the growth rates attainment of sexual maturity, reproduction and behavior (Hellawell 1986; Shaddock 2005).

According to Evins & Greaves (1979), in their studies conducted at Castle Carrock, Staines and Fobney, filter media appeared to play a role in the removal of invertebrates in rapid sand filters, in which sand of 0.5–1 mm in diameter was used, resulting in an effective pore size of 100–150 mm. Another investigation was conducted at Shenzhen city, Southern China (Liu 2010). After comparing the abundances of invertebrates in filtrate of two rapid sand filters, which had the same physical characteristics but different sand sizes, it was found that a higher removal ratio of invertebrates was obtained for the filter with smaller sand sizes. In highly clogged slow sand filters, faunal abundance significantly decreased with depth (Mauclaire *et al.* 2006). The results obtained from the current pilot-scale study (Table 3 and Figure 2) support the above views. The mean value, maximum value and standard deviation of invertebrates' abundance in filtrate of column 4#, which had a deeper sand bed and smaller sand sizes, were all the smallest.

Backwashing and its effect

Adam *et al.* (1998) indicated that invertebrates and Diptera levels could be maintained within the recommended limits (<20 ind./m^3 and <1 ind./m^3 respectively) in 'new' rapid sand filters, which were operated under a more advanced backwashing. Pilot-plant studies conducted by Steynberg *et al.* (1996) showed that proper backwashing of the sand filters was more important than small differences in media characteristics. Furthermore, Schreiber *et al.* (1997) indicated that the number of organisms in the filtrate was reduced by 80% with optimized backwashing procedures, which included 9 m/h pressure air for 15 min, 6.8 m/h drinking water for 10 min, 33.2 m/h drinking water for 20 min and filter to waste (16.8 m/h) after backwashing for 10 min. Therefore, although there was no backwashing executed in the present experiment out of consideration for supplying ideal habitats for invertebrates, it is necessary to backwash the BAC filters in normal operation to prevent the cumulative penetration of invertebrates (as shown in Figure 3), as BAC filters should become more productive with age according to the general theory of ecological succession (Engstrom *et al.* 2000). In the present study, with the sand bed added, three-layers of filtering including GAC layer, sand layer and gravel layer are formed. As a result of the backwashing operation, there was a significant phenomenon of a mixed layer between the GAC layer and sand layer. Sand would penetrate upwards to the GAC layer with air backwashing, while the GAC particles are drawn into the sand layer with the high velocity of backwashing water. These two cases are called 'infiltration of mixed layer' and 'involution of mixed layer' here. The interception efficiency of invertebrates will reduce in both cases, because the infiltration of mixed layer will lead the sand bed thin, while involution of mixed layer will make the sand bed uniformity coefficient increase. However, it was indicated that the good layering could be recovered by 20–30 m/h water backwashing for less than 2.5 min. Further work is needed to combine the backwashing and sand bed for more effective removal of invertebrates.

Filtrate quality – particulate matter removal

WHO (2004) pointed out that long-term control measures should be recommended to prevent animals reaching nuisance levels or, following disinfestation, to prevent recurrence of problems. The principal objective is to deny the animals a food supply and to restrict their entry into the distribution system. Probably the most important single step in limiting animal populations in mains is to minimize the quantity of particulate organic matter entering the distribution system. In the current study, with the sand beds added the particle counts in the filtrates can be removed effectively (as shown in Tables 4 and 5 and Figure 4(a)). The particles which were removed by the sand beds may include biofilm shedding, the decay of animals and their feces, which were easily available for the bacteria and small animals breeding. It is therefore inferred that the biological stability of filtrate of BAC filters can be improved by adding sand beds.

Comparison with other processes for removal of invertebrates

Micro-strainers are usually used as a pre-treatment to reduce solids loading before slow sand filters or chemical coagulation. When applied for the removal of zooplankton, micro-strainers are located either at the beginning or end of the treatment process. A coarser mesh of 200 μm aperture is sometimes used after GAC filters to remove eroding particles of the carbon and any bacterial flora or zooplanktons that sometimes develop in GAC filters (Ratnayaka *et al.* 2009). The coarser mesh is not good enough to removal the smaller rotifers and nemeous organisms such as nematodes, while a fine mesh clogs more easily and needs to be cleaned frequently. Another helpful process for the effective removal of invertebrates is to ensure the GAC filter is followed by a slow or rapid sand filter. However, for both the micro-strainer and sand filter located behind the GAC filter, extra space and sets of automatic equipments are required which may be inconvenient and increase the cost.

CONCLUSION

1. An over 300 mm height of sand bed (sand grading: 0.6–1.0 mm) was required to remove more than 40% of rotifers and nearly 80% of larger invertebrates (size >200 μm) for BAC filtration column. With the increasing depth of sand beds, removal ratios of larger invertebrates significantly increased. Sand size had a greater impact on rotifers removal than larger invertebrates.
2. With the sand beds added in BAC filters, the peak abundance and peak numbers of invertebrates in the filtrate could be reduced significantly during a long-running time.
3. The mean values of particle counts in the filtrates decreased gradually with the increasing depth of sand beds, which would help to deny invertebrates' food supply and thereby prevent recurrence of problems.
4. Backwashing was necessary to eliminate the excessively breeding invertebrates in normal operation because the cumulative penetration of invertebrates would happen inevitably with an increase running time. The mixed-layer phenomenon could be controlled under the optimized backwashing procedures.

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