Chemosphere 161 (2016) 43–52

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Exploring the biological stability situation of a full scale water distribution system in south China by three biological stability evaluation methods

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HIGHLIGHTS

- Correlation between BRP and AOC was significant in either WTP or DWDS.
- Correlation between BRP and BDOC was more significant in WTP than in DWDS.
- High chlorine residuals largely suppressed bacterial regrowth in DWDS.
- Low chlorine residuals led to a positive correlation between AOC/BRP and HPC.
- Heterotrophic bacteria were limited compared to that when AOC was more than 135 μg/L.

ARTICLE INFO

Article history:
Received 10 March 2016
Received in revised form
19 May 2016
Accepted 26 June 2016
Available online 13 July 2016

Handling Editor: Shane Snyder

Keywords:
Bacterial regrowth potential (BRP)
Assimilable organic carbon (AOC)
Biodegradable dissolved organic carbon (BDOC)
Biological stability evaluation
Full scale water distribution systems
Disinfectant effect

ABSTRACT

Bacterial regrowth especially opportunistic pathogens regrowth and contamination in drinking water distribution systems (DWDS) have become an emerging threat to public health in the whole world. To explore bacterial regrowth and biological stability, assimilable organic carbon (AOC), biodegradable dissolved organic carbon (BDOC) and bacterial regrowth potential (BRP) were evaluated in a full scale DWDS and bench tests in South China. A significant correlation between BRP and AOC in both water treatment processes (WTP) and DWDS was obtained. For BRP and BDOC, the correlation was more significant in WTP than in DWDS. Both AOC and BRP were significantly correlated with UV_{254}, total organic carbon (TOC), and heterotrophic plate count (HPC) ($p < 0.01$), whereas BDOC was only significantly associated with UV_{254}, temperature and chlorine residual ($p < 0.01$). Through a bench test, when chlorine was higher than 0.5 mg/L, the HPC level was low and AOC concentration almost unchanged. On contrary the HPC level increased quickly and declined slightly, with chlorine lower than 0.15 mg/L, which was in accordance with the large amount of biological stability data obtained from DWDS. Through another bench test, the HPC level was positively correlated to AOC concentration and when AOC was below 135 μg/L, the growth rate of HPC was low, which was verified by the analysis of biological stability data from DWDS.

1. Introduction

Bacteria in drinking water, especially opportunistic pathogens, are of growing concern. Water-borne pathogens in DWDS can cause many types of diseases such as acute gastro-intestinal disorder (Wang et al., 2012, 2014a; Huang et al., 2014). Unwanted or excessive bacterial growth in DWDS can cause deterioration of microbial water quality during storage and transport (Prest et al., 2016). Growth of bacteria on the inner pipe surface leads to the biofilm formation, deteriorate water quality, and induce public health issues (Wingender and Flemming, 2004; Ohkouchi et al., 2011; Park et al., 2012; Liu et al., 2013). Biological stability, also known as the tendency of microbial growth supported by water and/or contact material, has been extensively investigated for years

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http://dx.doi.org/10.1016/j.chemosphere.2016.06.099
0045-6535/

(Liu et al., 2013). Greater biological stability could be achieved with fewer nutrients present in water (Lai et al., 2006; Lou et al., 2012; Van der Kooij et al., 1989). One approach to assess biological stability is to quantify bacterial biomass (colony-forming units or CFU), such as bacterial regrowth potential (BRP) (Sathasivan, 1999). Another alternative suggestion is to quantify the changes of nutrients present in water, for instance assimilable organic carbon (AOC[1]) (van der Kooij et al., 1982), biodegradable dissolved organic carbon (BDOC) (Servais et al., 1987) in Table 1. Microbial growth in distribution systems is affected by water quality variations and multiple interactions between water, biofilms and sediments. Number of methods has been developed recently to improve the assessment and monitoring of the biological stability of drinking water (van der Kooij et al., 2015; Prest et al., 2016).

In particular, AOC has been considered as an indicator that is directly related to bacterial regrowth during water distribution (Yu et al., 2011; Lautenschlager et al., 2013; Wang et al., 2014b). AOC typically comprises only a small fraction (0.1%–9.0%) of total organic carbon (TOC) (Escobar and Randall, 2001). This is determined by monitoring the changes of organics using two different bacterial species (*Pseudomonas fluorescens* P17 and *Spirillum* NOX) (van der Kooij et al., 1982; Van der Kooij and Hijnen, 1984), which have different substrate specificities. The AOC measurement is performed with specific organics such as acetate and oxalate as the sole substrate, which may lead to different yield of biomass depending on bacterial strains. The AOC level of 10 μg C/L or less was proposed as the indicator for biologically stable drinking water in Netherlands (Van der Kooij, 1992a, 1992b). BDOC is the fraction of DOC that can be assimilated and/or mineralized by heterotrophic bacteria. Biodegradability of DOC is determined through measuring the DOC consumption by microorganisms (Servais et al., 1987). BDOC has been routinely used in water industry laboratories to indicate the quality of drinking water and also to serve as a measure of biological stability (Huck, 1990; Kaplan and Rice, 1994: van der Kooij and van der Wielen, 2013). Some reported values of BDOC and AOC used as measures of biological stability are compared in Table 2.

Despite their prevalence, these indicators are still limited by the assumption that bacterial growth is associated only with organic carbon sources (van der Kooij, 2000). The complex interactions (competition, antagonism, symbiosis, and commensalism) among the diverse bacterial strains in actual environments are not resolved by the AOC assessment (Sathasivan, 1999). Therefore, BDOC and AOC may not sufficiently and efficiently represent the actual organic carbon levels and bacterial growth in water. The BRP method relies on the total bacterial count of a water sample to indicate biological stability (Sathasivan, 1999). BPR is achieved by inoculating water samples with indigenous bacteria (Dixon et al., 2012), followed by the incubation and the measurement of total bacteria counts on R_2A agar. Indigenous bacteria can utilize a much broader and diverse kinds of organic carbon than a single pure culture and it enables a more realistic interpretation of the actual microbial activity (Hammes and Egli, 2005).

Water is generally recognized as relatively biologically stable if its AOC concentration ranges from 50 to 100 μg/L (LeChevallier et al., 1993; LeChevallier et al., 1996). In order to maintain water in DWDS biologically stable, it must have an appropriate level of chlorine residual (LeChevallier et al., 1996) or 10–20 μg/L of AOC concentration without chlorine (van der Kooij, 2000).

Heterotrophic plate count (HPC) was often used as an indicator of the drinking water quality (Uhl and Schaule, 2004; Liu et al., 2015) (Franson, 1995; WHO, 2011). The limit was set to 500 CFU/mL for the HPC (R_2A) 7-day incubation. Escobar et al. (Escobar and Randall, 2001) showed a strong positive association between the HPC and AOC, and a weak positive correlation between the HPC and BDOC. Carter et al (Carter et al., 2000). demonstrated no significant correlations between AOC and HPC; while Zhang and DiGiano (Zhang and DiGiano, 2002) observed weak negative to weak positive correlations among these factors. For which, it indicated that a high residual chloramine (>2 mg/L) could effectively repress microbial activity in waters even at high AOC levels. The reason for different results achieved by different researchers was that the interaction between the promotion of nutrients to support bacterial regrowth and the inhibition of chlorine residual to limit bacterial regrowth need to be considered together.

No existing method is capable of reflecting the biological stability completely. In China, most research studied on AOC levels in WTP and DWDS(W.J. Liu et al., 2000), few studies took BDOC and other indicators to reflect biological stability of drinking water based on a large amount of biological stability continuous monitoring data. Furthermore, few studies compared the interrelationship or correlations between these different evaluation methods of biological stability based on monitoring data in previous research. We performed a comparative study on biological stability of a full scale water distribution system in south China using AOC, BDOC, and BRP indicators and explored the correlations between them. The research is expected to (i) evaluate the biological stability in a full-scale DWDS; (ii) explore the correlation among BRP, AOC, BDOC and water quality; (iii) reveal the interaction mechanism among AOC, HPC and chlorine and disinfectant effect on bacterial regrowth in DWDS.

2. Materials and methods

2.1. Preparation of carbon-free materials

Carbon-free bottles and vials were prepared using the method of Hammes (Hammes et al., 2010). All glassware was first washed with detergent and rinsed three times with deionized water. Then, the glassware was submerged overnight in 0.2 N HCl and subsequently rinsed with deionized water again and then air dried. Finally, the bottles were baked in a Muffle furnace at 500 °C for at least 6 h. Teflon-coated screw caps for the glassware were washed and treated with 0.2 N HCl.

2.2. Layout of full-scale drinking water treatment plant and sampling sites

As is shown in Fig. 1, effluent water samples collected from treatment train of two water treatment plants (WTPs) and twelve sampling sites of the water distribution system in south China were chosen. The first WTP (WTP1) is conventional (i.e., pre-ozonation coagulation, flocculation, filtration, treated water) and the second WTP (WTP2) employs advanced processes (i.e., pre-ozonation coagulation, flocculation, filtration, ozonation and bacterial activated carbon (BAC)-filtration treated water). The operational parameters of two WTPs were shown in Table S1. Twelve sampling sites of the water distribution system were chosen. The sites 1 to 5 were from the conventional WTP1, while the sites 6 to 12 were from the advanced WTP2. Characteristics of sampling sites in DWDS can be seen in Table S2. Raw water and the effluent of the pre-ozonation contact tank (a horizontal sedimentation tank), sand

[1] Abbreviations AOC: assimilable organic carbon; BDOC: biodegradable dissolved organic carbon; BRP: bacterial regrowth potential; HPC: heterotrophic plate count; CFU: colony-forming units; TOC: total organic carbon; DWDS: drinking water distribution system; WTP: water treatment plant; RW: raw water; POCR: pre-ozonation contact reactor; CST: coagulation sedimentation tank; SF: sediment filtration; OCR: ozonation contact reactor; BAC: bacterial activated carbon; FW: finished water.

Table 1
Comparison of measurement methods for BRP, AOC, and BDOC.

Method	BRP	AOC	BDOC
Inoculation	Indigenous bacteria	Pure bacteria	Indigenous bacteria
Incubation temperature	20 °C	20 °C	22 °C-25 °C
Incubation time	5–7 d	3–5 d	28 d
Results	Total microbial count	Total microbial count	Difference between initial DOC and final DOC
Expression	Cell per milliliter	Acetate carbon	Organic carbon

Table 2
Reported biological stability guidelines for AOC and BDOC.

	Reported value	Literature	Reported value	Literature
AOC (without chlorine)	2–311 μg/L	Hammes et al. (Hammes et al., 2010)	32 μg/L	Hammes et al. (Hammes et al., 2010)
	4–130 μg/L	Ross et al. (Ross et al., 2013)	10 μg/L	Van der Kooij et al. (van der Kooij, 1992a, 1992b)
			4.6 and 5.3 μg/L	Boe-Hansen et al. (Boe-Hansen et al., 2002)
AOC (with chlorine)	92–482 μgC/L	Liu (Liu et al., 2002)	50–100 μg/L (>0.1 mg/LCl_2)	Lechevallier et al. (LeChevallier et al., 1987; LeChevallier et al., 1992; LeChevallier et al., 1993)
	36–446 μgC/L	Thayanukul (Thayanukul et al., 2013)	10.9 μg/L (0.05 mg/LCl_2) 33.6 μg/L (0.1 mg/LCl_2)	Yumiko Ohkouchi et al. (Ohkouchi et al., 2013)
	Average AOC concentration was 174 μgC/L in winter and 60 μgC/L in summer	Yumiko Ohkouchi (Ohkouchi et al., 2011)	200 μg/L (In China)	HongWei Wu (H.W. Wu and Zhang, 1999; W.J. Liu et al., 2000)
BDOC	<0.1–1 mg/L, typically 0.24–0.32 mg/L	Kaplan et al. (Kaplan et al., 1994), Volk and LeChevallier (Volk, 1999)	0.15 mg/L 0.15 mg/L at 20 °C; 0.30 mg/L at 15 °C	Servais et al. (Laurent et al., 1997) Volk (Volk, 2000)
		Bachmann and Edyvean (Bachmann and Edyvean, 2005)	0.25 mg/L	Niquette et al. (Niquette et al., 2001)

filter, ozonation contact tank, biological activated carbon (BAC) filter, and the pumping station in WTP2 were also collected to evaluate biological stability of effluent from different water treatment processes.

2.3. Water quality analysis

Water quality measurements include temperature, residual chlorine by HACH Pocket Colorimeter II, ammonia nitrogen and UV_{254} by Shimadzu UV2550 spectrophotometer, TOC by ligni TOC trace, turbidity (NTU) by HACH 2100AN and COD_{Mn} by Titration.

2.4. Biological stability evaluation

AOC method was determined with an assay which was described by Liu et al. previously (Liu et al., 2002). Briefly, within 6 h after sampling, water samples were filled into 40 mL sample tubes and heated at 70 °C for 0.5 h in order to destroy vegetative cells. After disinfection, the tubes were allowed to cool to room temperature before inoculation. Meanwhile, the samples were dechlorinated with 100 μl of 0.1 M thiosulfate solution. *P. fluorescens* strain P17 was inoculated into the water samples at a final concentration of 10,000 CFU/mL. It was followed by the incubation at 25 °C for 3 days and counting CFU to determine the bacterial growth. Subsequently, the same water samples were heated at 70 °C for 0.5 h to remove P17 before inoculating NOX strain. The inoculated water samples were incubated at 25 °C for 4 days with CFU again. The AOC concentrations were calculated by comparing the CFU numbers and yield coefficients (Liu et al., 2002). Positive or reference control experiments used 100 μg/L sodium acetate with P17 inoculum that generally yielded AOC of 83.70 ± 16.00 μg/L as acetate-C, while with NOX, AOC was determined to be 79.50 ± 11.30 mg/L as acetate-C. Biomass yield using the acetate standard was 1.1×10^7 CFU/μg of acetate-C for P17 and 1.7×10^7 CFU/μg of acetate-C for NOX, which agreed well with the referenced values as specified in the Standard Methods (van der Kooij et al., 1982; Van der Kooij and Hijnen, 1984).

BDOC analysis was performed by the procedure proposed by Servais et al (Servais et al., 1987). with minor modification. The water samples were first filtered using microfilter, the filtered water samples were distributed as 250 mL portions into 250 mL glass tubes. Thiosulfate was added to remove residual chlorine prior to inoculation with 2.5 mL of Taihu lake water with bacterial inoculum measuring 4.5×10^5 CFU/mL. For each analysis, twelve 250-ml tubes were required. The initial DOC concentrations in the tubes (parallels) were measured. After that the tubes were incubated in the dark at 20 °C without agitation. Determination of optimal growth time of BDOC is shown in Fig. S1 as supporting information (SI). On day 28, the change between DOC_0 and DOC_{28} was due to the biodegradation of organic carbon compounds. Additional controlled experiments included ultrapure water blank and DOC standard solutions.

BRP measurement followed the protocol developed previously (Sathasivan, 1999). The BRP method, simply a bioassay, has the following three basic steps; sample preparation, inoculation, and maximum biomass measurement. A 100 mL of water sample was collected into a test tube, continued by heating it at 70 °C for 30 min. Indigenous inoculum came from the raw water and was filtered through (pre-washed with 300 mL MilliQ, 18MΩ, water) 2.0 μm poly-carbonate membrane filter and incubated at 20 °C for a few (normally 5) days until the maximum was reached. After that, indigenous inoculum was added to the water samples and all samples incubated at 20 °C. When the amount of biomass reached its peak, it is then qualified to indicate the BRP (Dixon et al., 2012). Direct total microbial count was used for biomass measurement and results were expressed as CFU per milliliter, CFU/ml. HPC was

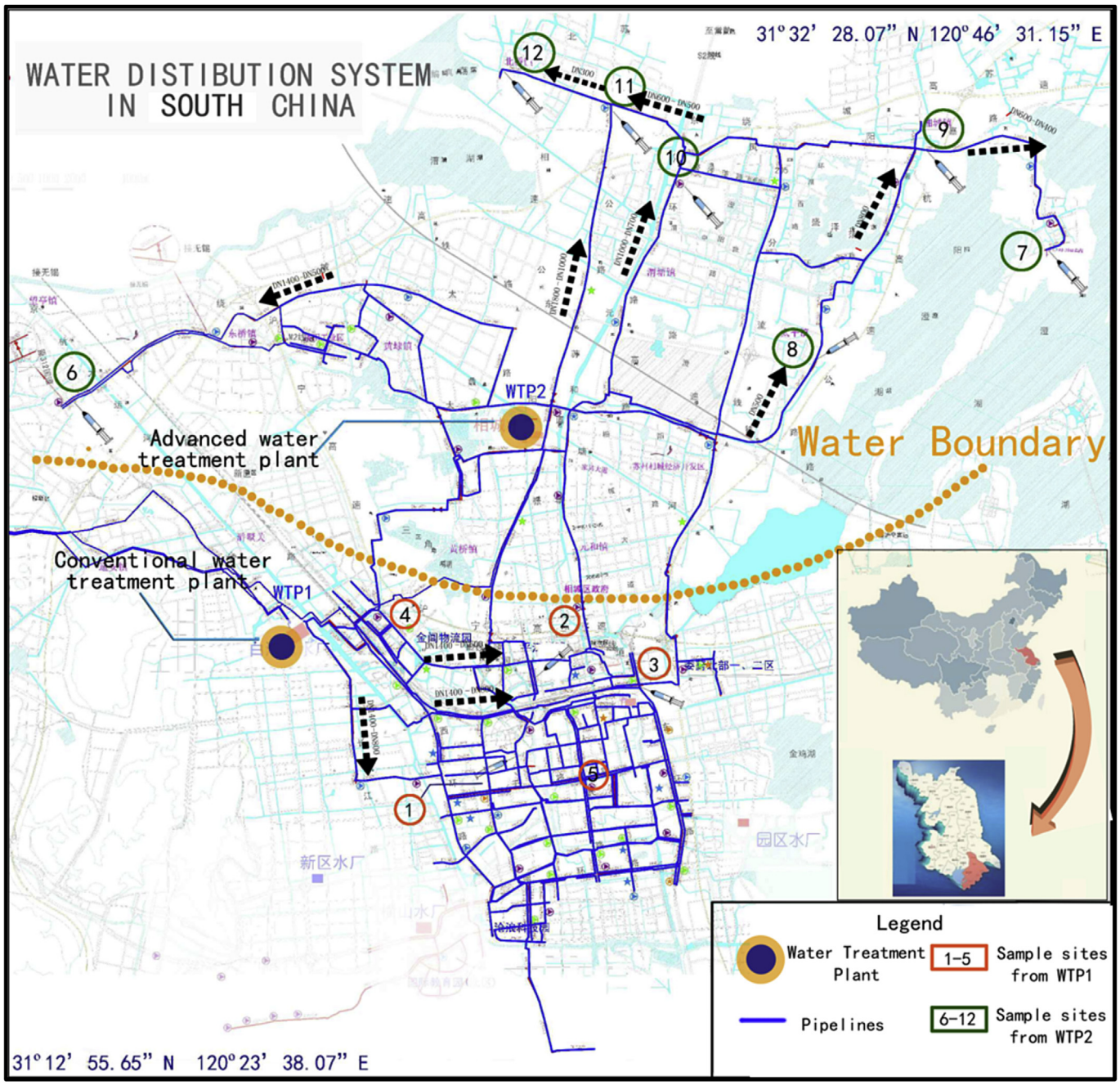

Fig. 1. Geographic locations of the two WTPs and the drinking water distribution in city of south China.

performed on R_2A agar at 22 °C for 7 d of incubation (Franson, 1995). The HPC for an untreated water sample was also determined when water sample was collected. Determination of optimal growth times of BRP is shown in Fig. S1 in the SI.

2.5. Bench test

For the first bench test, Drinking water samples were acquired from finished water from WTP2 in south China, and water was flushed for 15 min before sampling. 2 L of the sample was collected at initial free chlorine concentrations of 0.75 mg/L. Nothing was added to the sample, and it was incubated at 25° in the dark. Lastly, we measured the HPC, free chlorine, AOC and BRP at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 14, 28, 29, 30days.

For the second bench test, the bench test was developed to investigate the relationship between HPC and AOC in drinking water. Before test, the excess dosage of nutritional components was added to make the water samples of BRP values are not affected by the interference of other nutrients in the water, the nutrients and dosage were shown in Table S2. This test used BRP inoculum for inoculation, the inoculum was added to water samples and samples were incubated at 25° in the dark, the experiment did twice in different months.

2.6. Data presentation and calculations

Correlations were derived from linear regression analysis and expressed as Pearson's r values. Monitored data (12 months for

WTP1 and WTP2) was regressed using Origin 9.1 and SPSS version 18.0. A correlation was regarded as significant when the *P* value was below 0.01.

3. Results and discussion

3.1. Biological stability indicators

As was shown in Fig. 2, the biological stability of the full-scale DWDS was investigated all year round. BRP concentration ranged from 2.01×10^4 to 4.51×10^5 CFU/mL (Fig. 2a). BDOC concentration diverged within the range of 0.152–0.508 mg/L (Fig. 2c), and the AOC concentrations ranged from 40.5 to 307.9 g/L. The average values of AOC, BRP and BDOC were 106.6 μg C/L, 2.25×10^5 CFU/mL, 0.39 mg/L in the winter season and around 124.6 μg C/L, 2.16×10^5 CFU/mL, 0.38 mg/L in the summer season (Fig. 2b). The variations were mostly ascribed to water quality changes by different water treatment processes. After coagulation, sedimentation, and filtration, the BRP, AOC, and BDOC levels decreased. On the other hand, after main ozonation process, BRP, AOC, and BDOC increased slightly. After BAC, BRP, and BDOC decreased, AOC remained unchanged or declined slightly. Finally, after chlorination, BRP and AOC both increased. However, BDOC did not change significantly. The BRP levels of the raw water and finished water were almost equal, in contrast the AOC level rose and the BDOC level decreased. This result indicates that the water treatment process allowed the passage of AOC more than BDOC or that a significant proportion of new AOC were generated (e.g., oxidation products). Coagulation, sedimentation, filtration and BAC processes decrease AOC, BDOC and BRP levels; however oxidation processes (ozonation and chlorination) create AOC and BDOC, thereby increasing the overall bacterial growth potential of water (von Gunten, 2003; Hammes et al., 2006, 2007). The limited effect of oxidation processes on AOC removal was also noted, which was

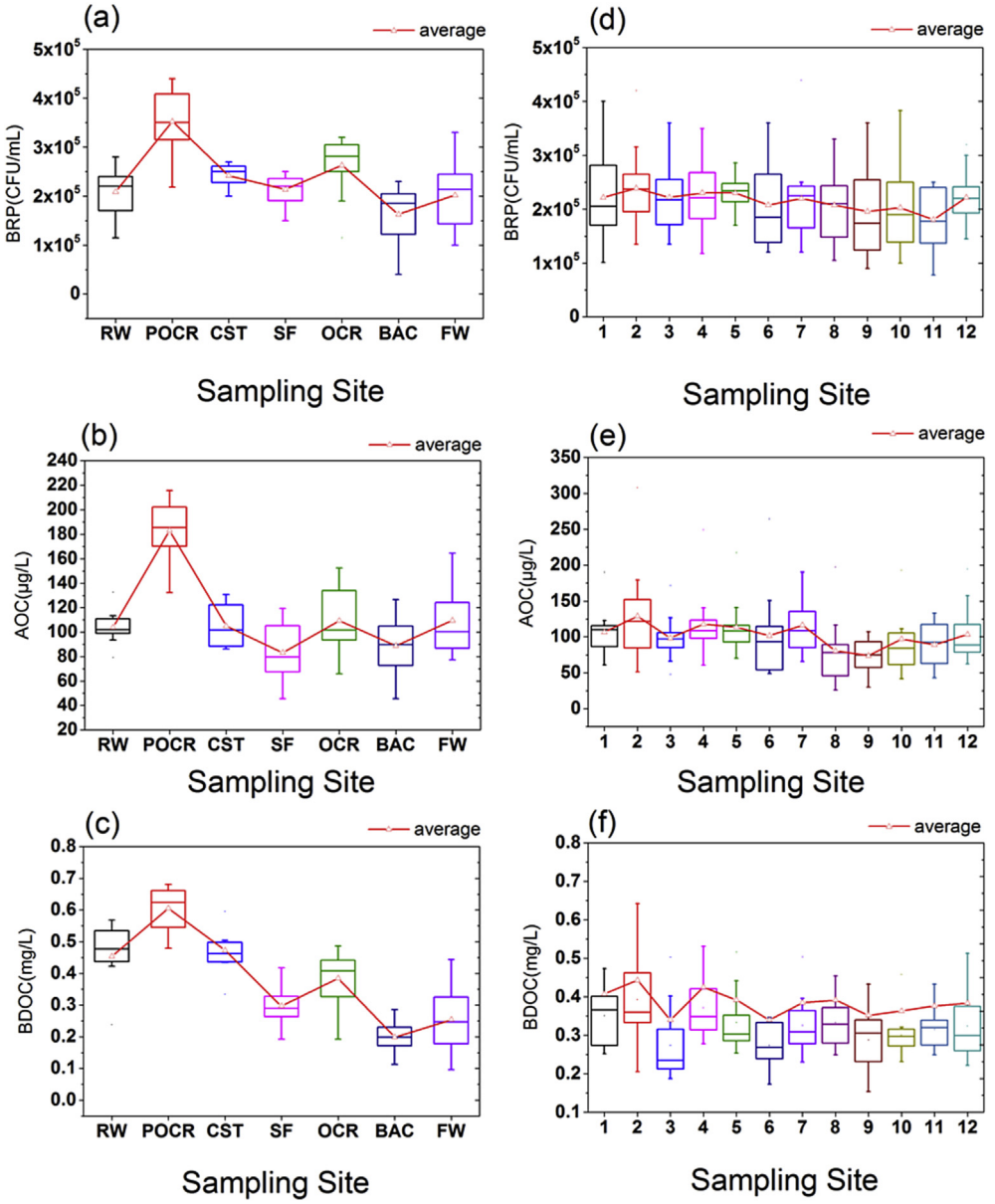

Fig. 2. Variation of different biological stability evaluation indicators during drinking water treatment process in different months Symbols: (a), (b), (c) were from WTP; (d), (e), (f) were from DWDS. RW (raw water), POCR (pre-ozonation contact reactor), CST (coagulation sedimentation tank), SF (sediment filtration), OCR (ozonation contact reactor), BAC (bacterial activated carbon), and FW (finished water).

consistent with the findings of Ohkouchi et al. (Ohkouchi et al., 2011).

At different sampling sites in DWDS, the BRP concentrations ranged from 8.01 × 10^4 to 4.45 × 10^5 CFU/mL with high levels in spring and autumn, and low levels in November (Fig. 2d). AOC concentration changed between 65.71 μg/L and 215.61 μg/L with the lowest level in October (Fig. 2f). The BDOC concentrations changed between 0.113 mg/L and 0.681 mg/L with high levels in September and October probably due to the sludge release in September in Taihu Lake (Fig. 2e). The AOC values in DWDS were higher than that of finished water. For sampling sites from 1 to 5 the average AOC level was higher than that of sampling sites from 6 to 12, which means the biological stability of water from advanced water treatment plant was higher than that from conventional water treatment plant. The obtained AOC values were much higher than the AOC level of 10 μg C/L proposed by van der Kooij (Van der Kooij, 1992a, 1992b) for biologically stable water in non-chlorinated systems. The AOC levels in DWDS were higher than 100 μg C/L, which was proposed as a criterion to prevent regrowth of coliforms in chlorinated systems by LeChevallier et al. (LeChevallier et al., 1996). As illustrated in Fig. 2, AOC and BRP showed a similar overall pattern either across WTP or in DWDS, although BDOC differed dramatically from the former two indicators.

3.2. Correlations between AOC, BDOC, and BRP and other water quality indicators

In the DWDS, the correlation between BRP and BDOC was not significant as shown in Fig. 3a ($r = 0.11$; $n = 144$; $p > 0.05$). Fig. 3b shows the interrelation between BRP and AOC to be significant ($r = 0.39$; $n = 144$; $P < 0.01$). Fig. 3c and d shows the relation between BRP and BDOC or between BRP and AOC were both significant for different water treatment processes ($p < 0.01$). Fig. 3e shows the association between AOC and BDOC to be significant for different water treatment processes ($r = 0.61$; $n = 144$; $p < 0.01$). The interaction between AOC and BDOC was not significant in

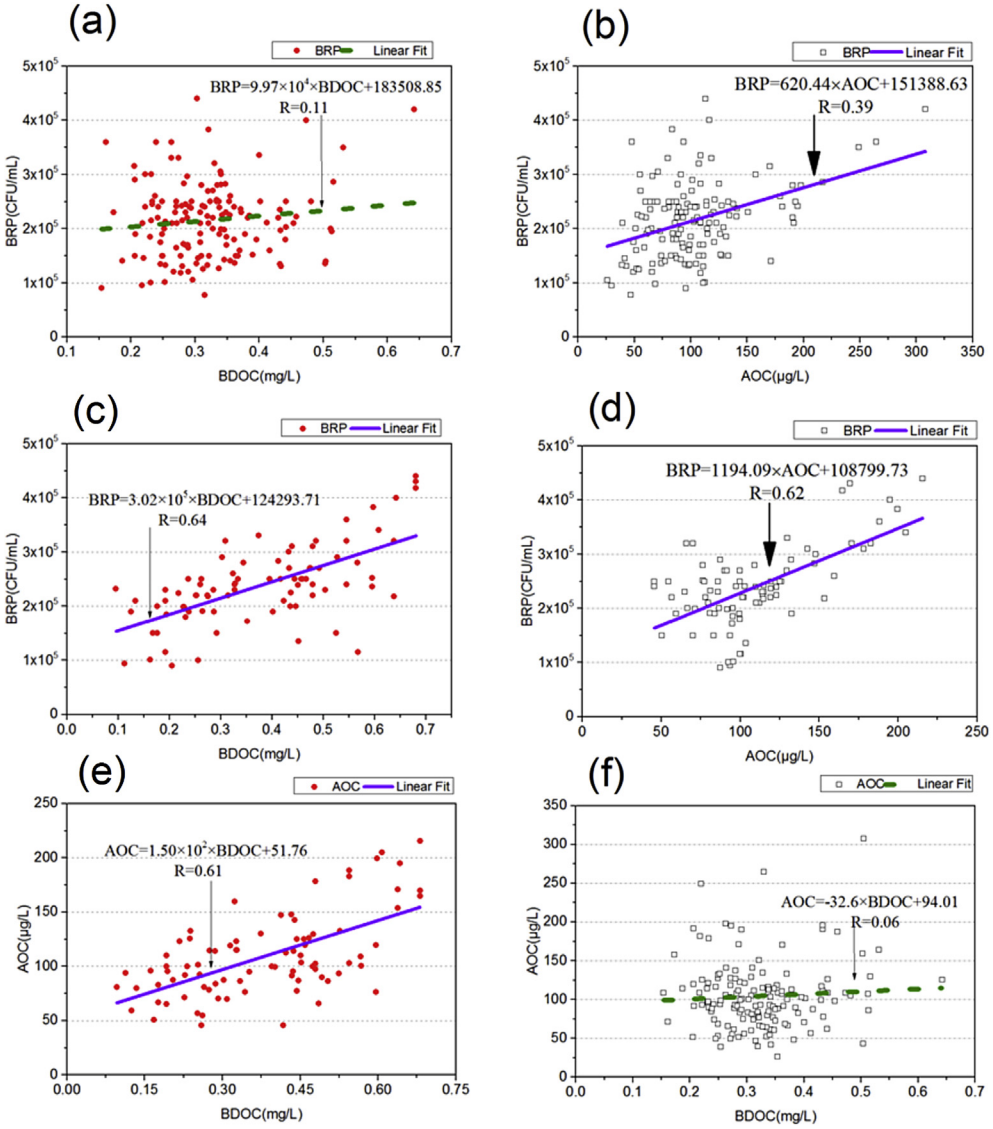

Fig. 3. (a) Correlation between BDOC and BRP and (b) between AOC and BRP in water distribution system (the twelve sampling sites) (c) Correlations between BDOC and BRP and (d) between AOC and BRP in WTP 2 (the seven sampling sites) (e) Correlations between AOC and BDOC in WTP 2 (the seven sampling sites) and (f) between AOC and BDOC in water distribution system (the twelve sampling sites). The solid line means the regression lines represent statistically significant correlations; the dash line means the correlations were not statistically significant.

DWDS ($r = 0.06$; $n = 144$; $p > 0.05$) as shown in Fig. 3f. Clearly, BRP well correlated with AOC in the full-scale water distribution system, while BRP could only associate with BDOC in water treatment processes. The positive relation obtained between the raw and drinking water samples supported that the water quality in DWDS is primarily governed by biological organic matter in raw water. As demonstrated in Fig. 3, for water distribution systems neither AOC nor BRP correlated with BDOC but a significant correlation was obtained between AOC and BRP. BDOC has detection limits that are at least an order of magnitude greater than AOC and BRP. Therefore, AOC and BRP might be effective and practical way to evaluate bacterial regrowth, which can reflect the water biological stability more flexibly. Table 1 summarizes the differences of three methods. Given the labor- and cost-intensive nature of each assay, a relevant issue is whether an assay alone is sufficient to indicate bacterial regrowth potential. BDOC and AOC quantify available carbon for bacterial growth, whereas BRP is a direct measurement of bacterial growth. As AOC is typically quantified by two specific strains of bacteria, P17 and NOX, AOC may better represent the specific groups of simple organic compounds (e.g., carboxylic and dicarboxylic acids). Van der Kooij (van der Kooij, 2000) suggested that BDOC could not predict the potential of bacterial regrowth because no significant correlation was found between this parameter and heterotrophic bacterial levels. The probable reasons are that the detection limit of the BDOC bioassay (0.1–0.2 mg/L) is too high for water samples. By contrast, the BRP method can reach lower detection limit of less than 100 μg/L of AOC as acetate-carbon. The experimental results demonstrated that BRP was similar to AOC as a simple operating method that detected bacteria regrowth potential sensitively. As for these three methods, AOC was widely used and suitable for different places, but difficult instrument operation and pure species preservation was the problem. To most WTP in China, it was a good method, but not all WTP was capable to detect it. BDOC has the same problem; it is time consuming and needs expensive equipment. BRP is easy to detect, but the relationship between BRP and AOC or BRP and BDOC should be calculated firstly for different region to reflect AOC levels or BDOC levels by detecting BRP.

Table 3 shows a number of significant statistical correlations between the sampling data. According to the statistical analysis of big data, both AOC and BRP were significantly associated with UV_{254}, TOC, and HPC ($p < 0.01$), whereas UV254, temperature and chlorine residual demonstrated significant association to BDOC ($p < 0.01$). HPC was significantly correlated with AOC, BRP, BDOC, COD_{Mn}, ammonia, free chlorine and temperature. The relationship between water quality parameters and biological stability evaluation methods showed that HPC, organic carbon and chlorine was the most important factors impact biological stability in DWDS. The relationship between different water quality parameters and biological stability evaluation methods are shown in Figs. S2–S4 as supporting information (SI).

3.3. Interaction mechanism among AOC, HPC and chlorine in DWDS

To explore particular biological stability situation of drinking water in South China, water samples were collected from water treatment plant and incubated at 25 °C without pasteurization, results showed that AOC concentration of treated water decreased quickly at first and reached to a stable situation after 30 days (Fig. 4). The treated water with chlorine disinfectant inhibited bacterial growth in the first four days with slight changes of AOC concentration and HPC concentration with chlorine residuals higher than 0.5 mg/L. The existence of a critical concentration in this range is in line with reports from other authors (Gillespie et al., 2014). Finished water or water with high free chlorine residual

Table 3
Correlation coefficients between the measurement data by BRP, AOC, and BDOC methods.

	AOC	BRP	BDOC
Turbidity (NTU)	−0.07	−0.02	**0.19***
COD_{Mn} (mg/L)	−0.10	−0.06	0.02
Ammonia nitrogen (mg/L)	−0.08	−0.15	0.12
UV_{254}	**0.28***	**0.25***	**−0.36***
Chlorine residual (mg/L)	−0.03	−0.07	**−0.19***
TOC(mg/L)	**0.29***	**0.26***	0.13
HPC(CFU/mL)	**−0.18***	**−0.18***	0.09
Temperature (°C)	−0.05	−0.01	**0.29***

A correlation coefficient of 1.0 (or −1.0) indicates a perfect relationship. A positive coefficient indicates that as one variable increases, so does the second. A negative correlation shows that as one variable increases, the second decreases.
*Means the correlation was significant.

displayed low proportions of intact cells, their relative numbers strongly increased in the distribution system when free chlorine residual dropped below 0.5 mg/L (Gillespie et al., 2014). From the fourth day to tenth day the chlorine declined quickly, and the AOC concentration of treated water decreased significantly and bacteria began to regrow, when chlorine was lower than 0.5 mg/L and higher than 0.15 mg/L, the result identified in this study is in very good agreement with guidelines. In low risk piped distribution systems, a free chlorine residual range of 0.2–0.5 mg/L should be maintained at all points in the supply (WHO, 1997) (Staff, 1997). From tenth day to thirtieth day the chlorine was less than 0.15 mg/L,

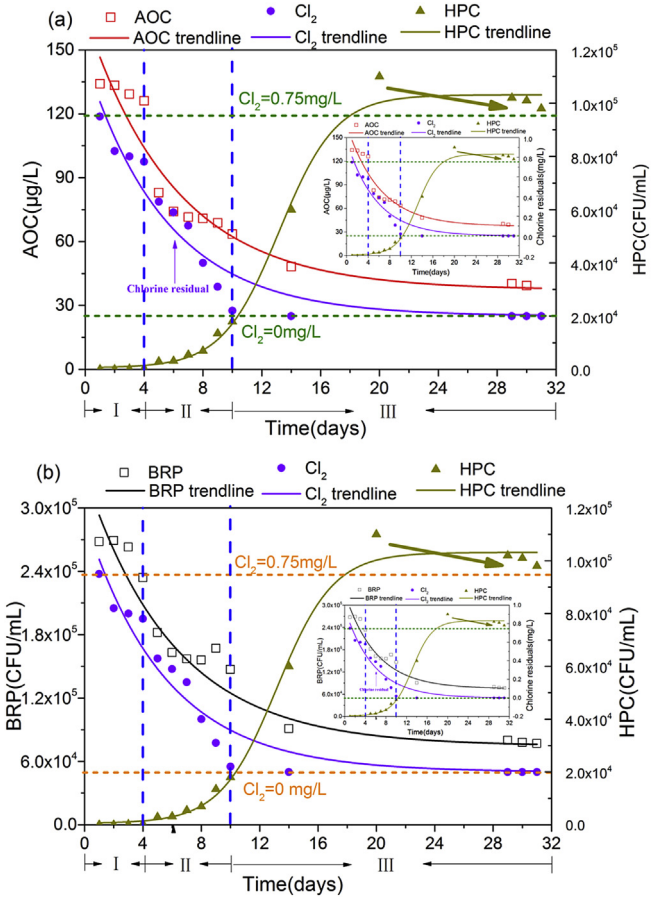

Fig. 4. Disinfectant effects on bacterial regrowth in a 30 days bench test. (a) Correlation between AOC and HPC; (b) Correlation between BRP and HPC in water distribution system (the twelve sampling sites).

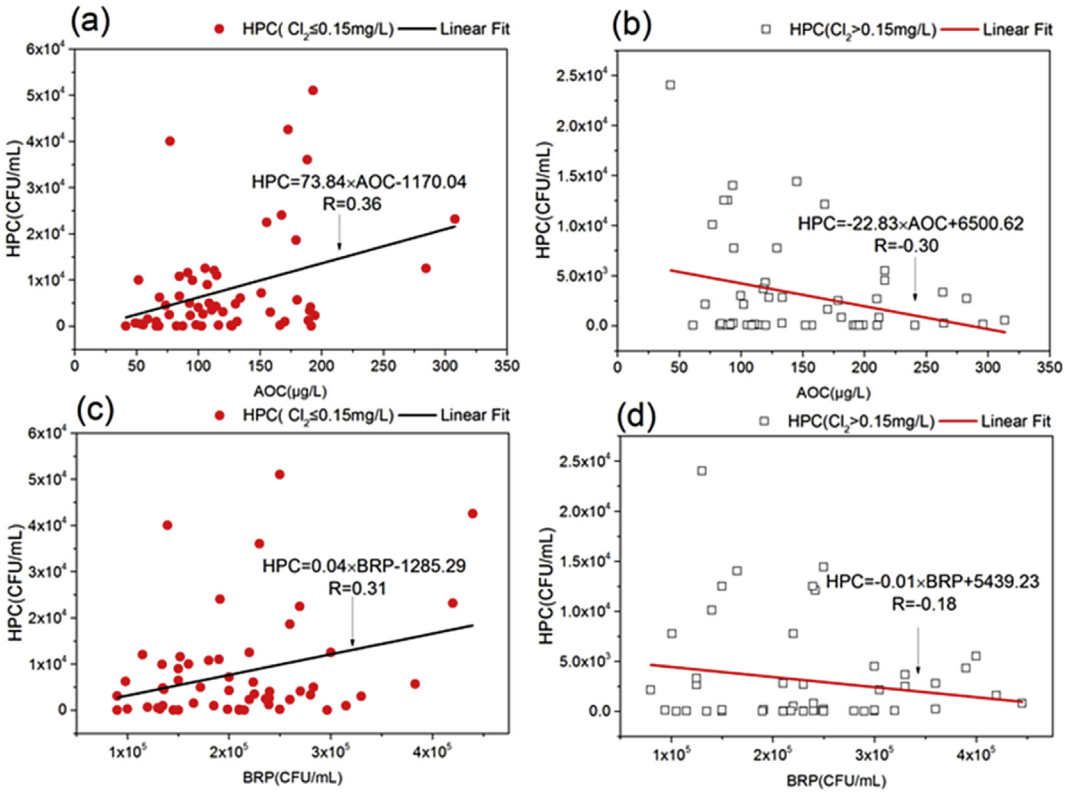

Fig. 5. (a) and (b) Chlorine residuals effects on AOC versus HPC in DWDS. (c) and (d) Chlorine residuals effects on BRP versus HPC in DWDS. The solid line means the regression lines represent statistically significant correlations; the dash line means the correlations were not statistically significant.

and HPC concentration increased quickly and decreased slightly after 20 days, while AOC concentration decreased. The variation of BRP concentration was also studied and it was similar to the variation of AOC in treated water within 30 days.

In the actual DWDS, the chlorine would interact with pipe material, biofilms and other components of drinking water as water flows, thus the chlorine residuals decreased fast and reaction among AOC, HPC and chlorine residuals would be accelerated. As a result, the reaction time of whole procedure would be shortened.

When chlorine residuals were higher than 0.5 mg/L in DWDS, the average HPC level was 216 CFU/mL, however, average HPC level was 6752 CFU/mL with chlorine less than 0.15 mg/L. The correlation between AOC and HPC in DWDS in this study was a weak negative interaction when chlorine residuals were more than 0.15 mg/L ($R = 0.10$; $p < 0.01$; $n = 72$) (Fig. 5) and HPC had a significant correlation with AOC when chlorine residuals were less than 0.15 mg/L ($R = 0.68$; $p < 0.01$; $n = 72$). This is probably because when chlorine residuals were more than 0.15 mg/L, the inactivation rate of bacteria by chlorine disinfectant could be equal to, or exceed the growth rate of bacteria in the samples which was also shown in bench test. Conversely, when chlorine residuals were less than 0.15 mg/L, the AOC tended to promote microbial growth. Similarly, approximately 81% and 93% of *Hartmannella vermiformis* were positive in biofilm and water samples, respectively, when the chlorination residual was ≤0.1 mg/L (Wang et al., 2012). Chlorination or chloramination treatments were reported to exert a strong selection process in the microflora in drinking water (Poitelon et al., 2010). The free chlorine concentration was an important factor for the variations in drinking water microflora (Hwang et al., 2012).

For the purpose of studying the relationship between AOC and HPC, pure water was used as water sample and nutrients were added to the water. Fig. 6 demonstrates that AOC levels greater than 135 μg/L led to high growth rate of HPC in the bench test. Therefore, to limit the AOC level less than 135 μg/L might be a proper guideline for DWDS. The result of bench test was verified by the analysis of the biological stability evaluation data obtained from DWDS. According to LeChevallier et al.'s statistical analysis method (LeChevallier et al., 1996) when free chlorine residual was less than 0.15 mg/L in water samples, AOC levels greater than 135 μg/L led to high levels of HPC. For which, HPC level was 2.89 times higher than that in chlorine-free systems with average AOC levels less than 135 μg/L. This AOC guideline concentration is lower than 200 μg/l

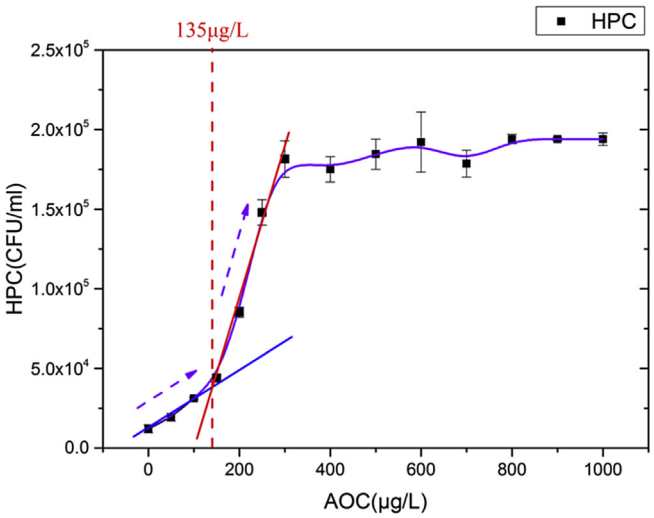

Fig. 6. Correlation of AOC and HPC in the second bench test.

proposed by Hongwei Wu(H.W. Wu and Zhang, 1999) in China and is a little bit higher than 50–100 μg/l put forward by LeChevallier (LeChevallier et al., 1996) in the U.S.A. The same relationship between BRP and HPC was achieved when BRP was less than 2.70×10^5 CFU/mL in this study. Water distribution systems disinfected by chlorination, with BRP levels greater than 2.70×10^5 CFU/ml had high level HPC concentration samples and 2.44 times higher HPC levels than free chlorinated systems with average BRP levels less than 2.70×10^5 CFU/ml. Unique conditions were possibly created with different chlorine residuals. Chlorine, which continuously reacted with organic compounds, might be the cause of the variations of drinking water properties. Water with high chlorine residual inhibited bacteria growth and fewer species were in the aquatic environment, which was more suitable for some slow-growing oligotrophs. Thus, when the amount of chlorine was high, indigenous bacteria lacked the ability to adapt to the aquatic environment, which differed from the situation with low chlorine residual.

4. Conclusions

Biological stability of a full-scale water distribution system in South China was detected by using three different evaluation methods (AOC, BDOC and BRP). High correlation was found between BRP and AOC in both WTP and DWDS ($p < 0.01$). Similarly, a significant association between BRP and BDOC in WTP was found ($p < 0.01$), but it was not significant in DWDS. As a result, BRP was similar with AOC as a simple and feasible bioassay that detected bacteria regrowth potential sensitively. Analysis of the sampling data showed that both AOC and BRP were significantly related with UV_{254}, TOC, and HPC ($p < 0.01$), whereas only UV_{254}, Temperature and Chlorine Residual demonstrated significant correlations to BDOC ($p < 0.01$). The relationship between water quality parameters and biological stability evaluation methods showed that the bacteria, organic carbon and chlorine were the most important factors impact biological stability in DWDS. Bench test showed that the interaction between the promotion of nutrients to support bacterial regrowth and the inhibition of chlorine residual to limit bacterial regrowth should be considered together in drinking water. When chlorine residuals were less than 0.15 mg/L, AOC and HPC had a positive correlation and promotion of AOC to support bacterial regrowth play a leading role. Subsequently, it had a negative correlation when chlorine residuals were more than 0.15 mg/L and inhibition of chlorine residual play a more important role. For drinking water with chlorine less than 0.15 mg/L, AOC and BRP less than 135 μg/L and 2.70×10^5 CFU/mL can limit heterotrophic bacteria regrowth.

Notes

The authors declare no competing financial interest.

Acknowledgments

We are grateful for the cooperation and participation of the utilities that were involved in this project, which is supported by National Key Technology R&D Program in the 12th Five year Plan of China (Project NO. 2012BAJ24B01) and Major Project of the Science and Technology Ministry in Suzhou China (Project NO. ss201434). We also appreciate Dr. Wen Zhang at NJIT for his advice and help on manuscript writing.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.chemosphere.2016.06.099.

References

Bachmann, R., Edyvean, R., 2005. Biofouling: an historic and contemporary review of its causes, consequences and control in drinking water distribution systems. Biofilms 2, 197–227.
Boe-Hansen, R., Albrechtsen, H.J., Arvin, E., Jorgensen, C., 2002. Bulk water phase and biofilm growth in drinking water at low nutrient conditions. Water Res. 36, 4477–4486.
Carter, J., Rice, E., Buchberger, S., Lee, Y., 2000. Relationships between levels of heterotrophic bacteria and water quality parameters in a drinking water distribution system. Water Res. 34, 1495–1502.
Dixon, M.B., Qiu, T., Blaikie, M., Pelekani, C., 2012. The application of the bacterial regrowth potential method and flow cytometry for biofouling detection at the Penneshaw Desalination Plant in South Australia. Desalination 284, 245–252.
Escobar, I.C., Randall, A.A., 2001. Assimilable organic carbon (AOC) and biodegradable dissolved organic carbon (BDOC): complementary measurements. Water Res. 35, 4444–4454.
Franson, M.A.H., 1995. American public health association American water works association water environment federation. Methods 6, 84.
Gillespie, S., Lipphaus, P., Green, J., Parsons, S., Weir, P., Juskowiak, K., Jefferson, B., Jarvis, P., Nocker, A., 2014. Assessing microbiological water quality in drinking water distribution systems with disinfectant residual using flow cytometry. Water Res. 65, 224–234.
Hammes, F.A., Egli, T., 2005. New method for assimilable organic carbon determination using flow-cytometric enumeration and a natural microbial consortium as inoculum. Environ. Sci. Technol. 39, 3289–3294.
Hammes, F., Salhi, E., Koster, O., Kaiser, H.P., Egli, T., von Gunten, U., 2006. Mechanistic and kinetic evaluation of organic disinfection by-product and assimilable organic carbon (AOC) formation during the ozonation of drinking water. Water Res. 40, 2275–2286.
Hammes, F., Meylan, S., Salhi, E., Koster, O., Egli, T., Von Gunten, U., 2007. Formation of assimilable organic carbon (AOC) and specific natural organic matter (NOM) fractions during ozonation of phytoplankton. Water Res. 41, 1447–1454.
Hammes, F., Berger, C., Köster, O., Egli, T., 2010. Assessing biological stability of drinking water without disinfectant residuals in a full-scale water supply system. J. Water Supply Res. T 59, 31.
Huang, K.L., Zhang, X.X., Shi, P., Wu, B., Ren, H.Q., 2014. A comprehensive insight into bacterial virulence in drinking water using 454 pyrosequencing and illumina high-throughput sequencing. Ecotoxicol. Environ. Safe 109, 15–21.
Huck, P.M., 1990. Measurement of biodegradable organic matter and bacterial growth potential in drinking water. J. Am. Water Works Assoc. 78–86.
Hwang, C., Ling, F.Q., Andersen, G.L., LeChevallier, M.W., Liu, W.T., 2012. Microbial Community dynamics of an urban drinking water distribution system subjected to phases of chloramination and chlorination treatments. Appl. Environ. Microbiol. 78, 7856–7865.
Kaplan, L.A., Rice, E.W., 1994. Survey of BOM in US drinking waters. J. Am. Water Works Assoc. 86, 121–132.
Kaplan, L.A., Reasoner, D.J., Rice, E.W., 1994. A survey of BOM in US drinking waters. J. Am. Water Works Assoc. 86, 121–132.
Lai, W.L., Yeh, H.H., Tseng, I.C., 2006. The effect of ozonation and filtration on AOC (assimilable organic carbon) value of water from eutrophic lake. Ozone Sci. Eng. 28, 29–35.
Laurent, P., Servais, P., Prévost, M., Gatel, D., Clément, B., 1997. Testing the SANCHO model on distribution systems. J. Am. Water Works Assoc. 89, 92–103.
Lautenschlager, K., Hwang, C., Liu, W.-T., Boon, N., Köster, O., Vrouwenvelder, H., Egli, T., Hammes, F., 2013. A microbiology-based multi-parametric approach towards assessing biological stability in drinking water distribution networks. Water Res. 47, 3015–3025.
LeChevallier, M.W., Babcock, T.M., Lee, R.G., 1987. Examination and characterization of distribution system biofilms. Appl. Environ. Microbiol. 53, 2714–2724.
LeChevallier, M.W., Becker, W.C., Schorr, P., Lee, R.G., 1992. Evaluating the performance of biologically active rapid filters. J. Am. Water Works Assoc. 84, 136–146.
LeChevallier, M.W., Shaw, N.E., Kaplan, L.A., Bott, T.L., 1993. Development of a rapid assimilable organic carbon method for water. Appl. Environ. Microbiol. 59, 1526–1531.
LeChevallier, M.W., Welch, N.J., Smith, D.B., 1996. Full-scale studies of factors related to coliform regrowth in drinking water. Appl. Environ. Microbiol. 62, 2201–2211.
Liu, W.J., Zhang, L.P., Wang, Z.S., 2000. The study on AOC bioassay in drinking water. Water Wastewater 26, 1–5.
Liu, W., Wu, H., Wang, Z., Ong, S.L., Hu, J.Y., Ng, W.J., 2002. Investigation of assimilable organic carbon (AOC) and bacterial regrowth in drinking water distribution system. Water Res. 36, 891–898.
Liu, G., Verberk, J.Q., Van Dijk, J.C., 2013. Bacteriology of drinking water distribution systems: an integral and multidimensional review. Appl. Microbiol. Biotechnol. 97, 9265–9276.
Liu, X., Wang, J., Liu, T., Kong, W., He, X., Jin, Y., Zhang, B., 2015. Effects of assimilable organic carbon and free chlorine on bacterial growth in drinking water. PloS One 10, e0128825.
Lou, J.C., Lin, C.Y., Han, J.Y., Tseng, W.B., Hsu, K.L., Chang, T.W., 2012. Comparing removal of trace organic compounds and assimilable organic carbon (AOC) at

advanced and traditional water treatment plants. Environ. Monit. Assess. 184, 3491–3501.
Niquette, P., Servais, P., Savoir, R., 2001. Bacterial dynamics in the drinking water distribution system of Brussels. Water Res. 35, 675–682.
Ohkouchi, Y., Ly, B.T., Ishikawa, S., Aoki, Y., Echigo, S., Itoh, S., 2011. A survey on levels and seasonal changes of assimilable organic carbon (AOC) and its precursors in drinking water. Environ. Technol. 32, 1605–1613.
Ohkouchi, Y., Ly, B.T., Ishikawa, S., Kawano, Y., Itoh, S., 2013. Determination of an acceptable assimilable organic carbon (AOC) level for biological stability in water distribution systems with minimized chlorine residual. Environ. Monit. Assess. 185, 1427–1436.
Park, S.-K., Choi, J.-H., Hu, J.Y., 2012. Assessing bacterial growth potential in a model distribution system receiving nanofiltration membrane treated water. Desalination 296, 7–15.
Poitelon, J.B., Joyeux, M., Welte, B., Duguet, J.P., Prestel, E., DuBow, M.S., 2010. Variations of bacterial 16S rDNA phylotypes prior to and after chlorination for drinking water production from two surface water treatment plants. J. Ind. Microbiol. Biot. 37, 117–128.
Prest, E.I., Hammes, F., van Loosdrecht, M.C., Vrouwenvelder, J.S., 2016. Biological stability of drinking water: controlling factors, methods, and challenges. Front. Microbiol. 7.
Ross, P.S., Hammes, F., Dignum, M., Magic-Knezev, A., Hambsch, B., Rietveld, L.C., 2013. A comparative study of three different assimilable organic carbon (AOC) methods: results of a round-robin test. Water Sci. Technol. 13, 1024–1033.
Sathasivan, A., 1999. Application of new bacterial regrowth potential method for water distribution system-a clear evidence of phosphorus limitation. Water Res. 33, 137–144.
Servais, P., Billen, G., Hascoët, M.-C., 1987. Determination of the biodegradable fraction of dissolved organic matter in waters. Water Res. 21, 445–450.
Staff, W.H.O., 1997. Guidelines for Drinking-water Quality: Surveillance and Control of Community Supplies. World Health Organization.
Thayanukul, P., Kurisu, F., Kasuga, I., Furumai, H., 2013. Evaluation of microbial regrowth potential by assimilable organic carbon in various reclaimed water and distribution systems. Water Res. 47, 225–232.
Uhl, W., Schaule, G., 2004. Establishment of HPC (R2A) for regrowth control in non-chlorinated distribution systems. Int. J. Food. Microbiol. 92, 317–325.
Van der Kooij, D., 1992a. Assimilable organic carbon (AOC) as an indicator of bacterial regrowth. Water Res. 84, 56–57.
van der Kooij, D., 1992b. Assimilable organic carbon as an indicator of bacterial regrowth. J. Am. Water Works Assoc. 84, 57–65.
van der Kooij, D., 2000. Biological stability: a multidimensional quality aspect of treated water. Water Air Soil Poll. 123, 25–34.
Van der Kooij, D., Hijnen, W.A., 1984. Substrate utilization by an oxalate-consuming spirillum species in relation to its growth in ozonated water. Appl. Environ. Microbiol. 47, 551–559.
van der Kooij, D., van der Wielen, P.W., 2013. Microbial growth in drinking-water supplies: problems, causes, control and research needs. Water Intell. Online 12, 9781780400419.
van der Kooij, D., Visser, A., Hijnen, W.A.M., 1982. Determining the concentration of easily assimilable organic carbon in drinking water. J. Am. Water Works Assoc. 74, 540–545.
Van der Kooij, D., Hijnen, W.A.M., Kruithof, J.C., 1989. The effects of ozonation, biological filtration and distribution on the concentration of easily assimilable organic-carbon (AOC) in drinking water. Ozone Sci. Eng. 11, 297–311.
van der Kooij, D., Martijn, B., Schaap, P.G., Hoogenboezem, W., Veenendaal, H.R., van der Wielen, P.W., 2015. Improved biostability assessment of drinking water with a suite of test methods at a water supply treating eutrophic lake water. Water Res. 87, 347–355.
Volk, C.J., 1999. Impacts of the reduction of nutrient levels on bacterial water quality in distribution systems. Appl. Environ. Microbiol. 65, 4957–4966.
Volk, C.J., 2000. Assessing biodegradable organic matter. J. Am. Water Works Assoc. 92, 64–76.
von Gunten, U., 2003. Ozonation of drinking water: part I. Oxidation kinetics and product formation. Water Res. 37, 1443–1467.
Wang, H., Masters, S., Hong, Y.J., Stallings, J., Falkinham, J.O., Edwards, M.A., Pruden, A., 2012. Effect of disinfectant, water age, and pipe material on occurrence and persistence of legionella, mycobacteria, pseudomonas aeruginosa, and two amoebas. Environ. Sci. Technol. 46, 11566–11574.
Wang, H., Masters, S., Edwards, M.A., Falkinham, J.O., Pruden, A., 2014a. Effect of disinfectant, water age, and pipe materials on bacterial and eukaryotic community structure in drinking water biofilm. Environ. Sci. Technol. 48, 1426–1435.
Wang, Q.H., Tao, T., Xin, K.L., Li, S.P., Zhang, W.F., 2014b. A review research of assimilable organic carbon bioassay. Desalin. Water Treat. 52, 2734–2740.
WHO, G., 2011. Guidelines for Drinking-water Quality. World Health Organization. 216, 303–304.
Wingender, J., Flemming, H.C., 2004. Contamination potential of drinking water distribution network biofilms. Water. Sci. Technol. 49, 277–286.
Wu, H.W., Zhang, S.Q., 1999. Study on removal of assimilable organic carbon in conventional water treatment process. China Water & Wastewater 15, 7–9.
Yu, W.Z., Gregory, J., Liu, T., Yang, Y.L., Sun, M., Li, G.B., 2011. Effect of enhanced coagulation by KMnO4 on the fouling of ultrafiltration membranes. Water Sci. Technol. 64, 1497–1502.
Zhang, W.D., DiGiano, F.A., 2002. Comparison of bacterial regrowth in distribution systems using free chlorine and chloramine: a statistical study of causative factors. Water Res. 36, 1469–1482.