



Effects of sterilization treatments on the analysis of TOC in water samples

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Abstract

Decomposition experiments conducted with and without microbial processes are commonly used to study the effects of environmental microorganisms on the degradation of organic pollutants. However, the effects of biological pretreatment (sterilization) on organic matter often have a negative impact on such experiments. Based on the principle of water total organic carbon (TOC) analysis, the effects of physical sterilization treatments on determination of TOC and other water quality parameters were investigated. The results revealed that two conventional physical sterilization treatments, autoclaving and ⁶⁰Co γ -radiation sterilization, led to the direct decomposition of some organic pollutants, resulting in remarkable errors in the analysis of TOC in water samples. Furthermore, the extent of the errors varied with the intensity and the duration of sterilization treatments. Accordingly, a novel sterilization method for water samples, 0.45 μ m micro-filtration coupled with ultraviolet radiation (MCUR), was developed in the present study. The results indicated that the MCUR method was capable of exerting a high bactericidal effect on the water sample while significantly decreasing the negative impact on the analysis of TOC and other water quality parameters. Before and after sterilization treatments, the relative errors of TOC determination could be controlled to lower than 3% for water samples with different categories and concentrations of organic pollutants by using MCUR.

Key words: sterilization; autoclave; ⁶⁰Co γ -radiation; micro-filtration; ultraviolet radiation

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Introduction

Total organic carbon (TOC) is a direct and reliable indicator that has been widely used to characterize organic pollution in water bodies. Specifically, TOC is commonly used to assess the environmental quality of aquatic systems, identify fatal pollution accidents, verify sources of environmental contamination and for monitoring and assessment of organic micro-pollution of drinking water (Black et al., 1996; Fu et al., 2003; Li et al., 2003; Huang et al., 2005; Katsoyiannis and Samara, 2007; Musikavong and Wattanachira, 2007; Batziaka et al., 2008; Carrasco et al., 2008; Llop et al., 2009). Recently, high-temperature oxidation (600–1000°C) and sensitive non-dispersive infrared absorption spectrometry detection technology (NDIR) for measurement of the CO₂ produced by sample degradation have become common method in determining TOC levels. Since 1973, several international communities have successively issued over 20 rules and guidelines for evaluating the levels of TOC in aqueous matrices (Visco et al., 2005). However, these methods have varied in their sensitivity, specificity, applicability, limitations and stability, as well as their susceptibility to interference, speed, simplicity and sampling range due to

differences in the oxidizing process and/or the detector used. Most methods used to evaluate TOC are designed to oxidize the organic matter in the water sample as thoroughly as possible to enable accurate measurement of the CO₂ produced by oxidation of the organic matter. Accordingly, prior to transfusing the water sample into the combusting tube for high-temperature oxidation, the sample must be filtered through 0.45 μ m millipore filters to remove suspended solid, so that high oxidation efficiency is attained and to ensure that the sampling tube does not become blocked.

The measurement of TOC does not include all organic carbon in a water sample, particularly with respect to larger particles of organic matter. The reason is that different organic molecules require different reaction conditions for complete oxidation due to their recalcitrance. Specifically, easily degradable molecules can be oxidized under mild conditions, while complete oxidation of recalcitrant compounds requires harsh conditions (Warnken and Santschi, 2004; Visco et al., 2005). Indeed, Leiviskä et al. (2008) demonstrated that the measured TOC for the 3 kDa fraction of organic matter of the same water samples was considerably higher than that of the 30 kDa fraction obtained by ultrafiltration (30 kDa and 3 kDa) of wastewater samples into different size fractions.

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Micro-organisms are extremely important in the purification of water in aquatic ecosystems due to their ability to decompose organic pollutants (Azam et al., 1983; Wang et al., 2002; González C et al., 2008; Mancera-López et al., 2008). Consequently, monitoring of TOC has been widely applied in studies conducted to evaluate the capacity of microorganisms to degrade organic pollutants (Gamage and Asaeda, 2005; Mrayyan and Battikhi, 2005; Radjenovic et al., 2007). Sterilization treatments are commonly employed to analyze the effects of microbial activity on the degradation and transformation of organic matter in water. By comparing variations in the level of organic pollutants (i.e., TOC) in samples with and without microbial activity, the decontamination potentials of microorganisms in aquatic systems can be evaluated.

Various methods of sterilizing water have been adopted based on thermal, chemical, physicochemical and electrochemical treatments. Steam sterilization has been widely used to evaluate the effects of biological activity on the release of P in aqueous systems (Dorozhkin et al., 2000; Jiang et al., 2008). Chloramphenicol has frequently been used as an antibiotic to evaluate the influence of bacteria on nutrient cycles of C, N and P (Clavero et al., 1999; Gamage and Asaeda, 2005). Recently, the photo-sterilization activity of TiO₂ has attracted a great deal of attention (Michael et al., 1999; Tamai et al., 2002; Ma et al., 2004). Radiation treatment and pulsed electric fields have also been widely studied and applied in water research (Skubko et al., 1976; Romantsev et al., 1991; Ohshima and Sato, 2004).

However, the addition of chemicals and/or antibiotics to water samples for sterilization will likely interfere with the measurement of TOC and other water quality parameters. For example, the use of large amounts of chloramphenicol to suppress bacterial activity will inevitably have a negative influence on the analysis of TOC and other corresponding water quality parameters. Conversely, the process of physical sterilization may transform the state of organic pollutants, which could lead to alterations of their species or physical and chemical properties. Although bactericidal efficacy can be guaranteed by physical sterilization techniques without the addition of any substances, the measured levels of organic pollutants immediately prior to and after physical sterilization may differ significantly, especially the measured TOC.

As a result, sterilization treatments may lead to large difficulties in evaluation of the effects of microbial activity on the degradation and transformation of organic material in aquatic systems. To ensure the reliability of experimental results, a successful sterilization method that has the following characteristics should be employed: (1) the entire sterilization process should not take long; (2) the sterilization methods should be applicable to typical types of contaminated water with different organic pollution intensities; (3) the bactericidal efficacy should be maintained for a long period; and (4) the sterilization methods should not have a large impact on TOC.

Despite the importance of the effects of sterilization, few studies have been conducted to evaluate the effects of sterilization treatments on the analysis of TOC in water

samples. Therefore, this study was conducted to illustrate the effects of several methods of conventional physical sterilization on the measurement of TOC in water samples, and to develop a novel sterilization method that is capable of a high bactericidal efficacy with low effects on the organic pollutants in the water sample. The developed method was also used to evaluate wide concentration ranges of TOC in water samples to demonstrate its reliability and applicability.

1 Materials and methods

1.1 Experimental materials

Water samples were collected from the urban reach of the Xinchang River (polluted by municipal sewage) and Huajiachi Lake (scenic water) in Hangzhou City, Zhejiang Province, China. Sterilization treatments and TOC/TN measurements were conducted immediately after sampling. All experiments were performed in transparent glass tubes. Three methods of sterilization were adopted (Table 1), autoclave sterilization at 121°C for 5–30 min under saturated steam (Autoclaver, YSQ.SG41.280, Medical Devices Company, Ningbo, China), ⁶⁰Co γ -radiation sterilization at a dose of 30 kGy (Institute of Nuclear Agricultural Science, Zhejiang University, China), and micro-filtration coupled with ultraviolet radiation (MCUR) using a 0.45- μ m cellulose acetate membrane (Xingya Purifying Material Company, Shanghai, China), and ordinary two-terminal ozonic ultraviolet germicidal lamp (40 W, Minghui Lighting-Source Factory, Haining, China). Experimental procedures were set up and conducted in an asepsis room. TOC determined by subtracting the TIC (total inorganic carbon) from the TC (total carbon) as shown in Fig. 1. Total nitrogen (TN) were measured using a Multi N/C-3100 analyzer at a burning temperature of 850°C (combustion-supporting gas: oxygen; catalyst: cerium oxide) in conjunction with NDIR detector and chemiluminescence detector (Analytik Jena AG Co., Germany), respectively. Potassium hydrogen phthalate and potassium nitrate/ammonium sulfate were used as external standards for TOC and TN quantification with relative standard deviations (RSD) of TOC \leq 1% and TN \leq 1.5%. Chl-*a* was measured using Hydrolab-DS5X Water Quality

Table 1 Sterilizing apparatus and main parameters

Sterilization treatment	Main parameter	Remark (glassware/water sample)
Autoclave sterilizer	Pressure: 1.05 kg/cm ² Temperature: 121°C Time: 5–30 min	Container: colorimetric tube Volume of water sample: 50 mL
⁶⁰ Co γ -radiation	Absorbed dose: 30 kGy	Container: Jar with a lid Volume of water sample: 1 L
MCUR	Microfiltration: 0.45 μ m Power: 40 W Density: 100–120 μ W/cm ² Wavelength spectra: (253.7 + 185.0) nm Time: 5–70 min	Container: transparent glass vessel Volume of water sample: 2.7 cm diameter \times 7.5 cm length

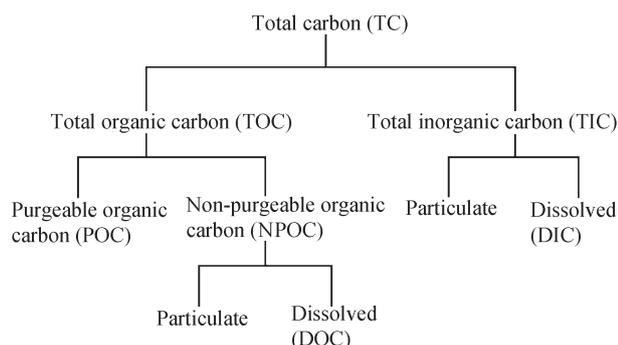


Fig. 1 Carbon in water; summary of the typical definitions and their relationships (Visco et al., 2005).

Multiprobes (HACH Company, USA).

1.2 Experimental setup

Figure 2 summarizes the procedures used for the sterilization treatments. Two treatments, T_A (without sterilization) and T_B (sterilization using either an autoclave, ^{60}Co γ -radiation or MCUR) were conducted in the present study, and all sterilization treatments were performed in triplicates. For the MCUR treatment, water samples were filtered through 0.45 μm millipore filters, and then the filtered water was sterilized under UV light with a set time of 5, 10, 15, 20, 25, 30, 40, 50, 60 or 70 min in well-transparent glass bottles that were 7.5 cm high and had a diameter of 2.7 cm. To determine the bactericidal efficacy, samples were serially diluted from 10^2 – 10^6 and then incubated at 37°C for 24 hr after each sterilization treatment in medium composed of 3 g/L beef extract, 10 g/L tryptone, 5 g/L NaCl, and 15 g/L agar (pH 7.0–7.2).

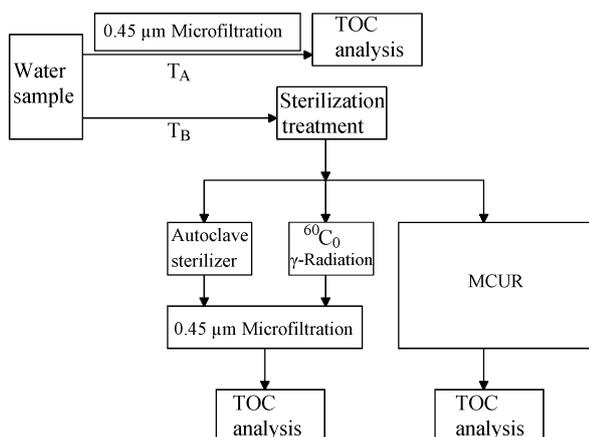


Fig. 2 Sampling and sterilization procedures used prior to TOC analysis.

2 Results and discussion

2.1 Sterilization efficacy

The bacterial culture experiments showed that the number of viable bacteria in T_A was approximately 10^5 – 10^6 and 10^2 – 10^3 colony-forming units (CFU)/mL before and after 0.45 μm millipore filtration, respectively (Huajiachi Lake). However, no bacteria were detected after sterilization was conducted using an autoclave or ^{60}Co γ -radiation.

Furthermore, when MCUR was conducted, no bacteria were detected after ultraviolet irradiation for more than 25 min (Fig. 4).

Even after pretreatment by 0.45 μm millipore filtration, some small individual micro-organisms with a size less than 0.45 μm remained in the sample, such as small bacteria, Rickettsia, mycoplasma, Chlamydia, Treponema and viruses (Table 2) (Wang and Shan, 2005). Although 0.45 μm millipore filtration can remove considerable amounts of micro-organisms from water samples, it cannot remove all microorganisms or inhibit the reproduction of remaining microbes.

2.2 Effect of autoclave sterilization on TOC

Autoclaving is widely applied for physical sterilization. According to the Evaluation Method and Standard for Efficacy of Disinfection and Sterilization issued by the Chinese Internal Bureau of Technical Supervision (GB 15981-1995), autoclaving at 121°C for 30 min at 1.05 kg/cm² is capable of sterilizing water samples completely. In the present study, different water samples with various categories and concentrations of organic pollutants were utilized to evaluate the effects of autoclaving for different time (5–30 min) on TOC, IC and TC. As shown in Fig. 3, the TOC and TC fluctuated sharply with treatment time. Specifically, for urban river water, the measured TOC and TC increased from 5.22 mg/L and 17.94 mg/L to 772.80 mg/L and 785.10 mg/L, respectively, after 15 min of treatment (Fig. 3a). For municipal scenic water (Fig. 3b), the measured TOC and TC also increased remarkably with sterilization time, although the variation in the ranges of these values was much smaller than the variation shown in Fig. 3a.

In a recent study conducted to evaluate the relationship between molecular mass distribution and binding affinity of organic ligands, Wu and Tanoue (2001) reported that the binding affinity of organic ligands increased as their molecular weight increased. Accordingly, the variations observed in our experiments may be due to the alteration of the conformations of some organic macromolecules by pyrolysis during autoclaving, which could result in non-flammable or incompletely combusted organic pollutants becoming more easily burned. The variation in the TOC and TC also changed with the intensity and duration of

Table 2 Classification and size distribution of microorganisms

Community	Particle size (diameter \times length) (μm)	
Prokaryotic microorganisms	Bacteria	(0.5–1.0) \times (0.5–5)
	Actinomycetes	0.2–1.2 (mycelium)
	Cyanobacteria	0.5–1.0
	Archaeobacteria	0.4–2.0
	Sheathe bacteria	> 1.0
	Rickettsia	(0.3–0.7) \times (1.0–2.0)
Eukaryotic microorganisms	Mycoplasma/Chl- <i>a</i> mydia	(0.2–0.25)/(0.2–0.3)
	Fungi	(0.1–3.0) \times (3–500)
	Micro-algae	(2.0–10) \times (5.0–30) (mycelium)
	Protozoan	> 2.0
Non-cellular microorganisms	Micro-metazoan	30–300
	Virus	40–2000
	Subviruses	< 0.3

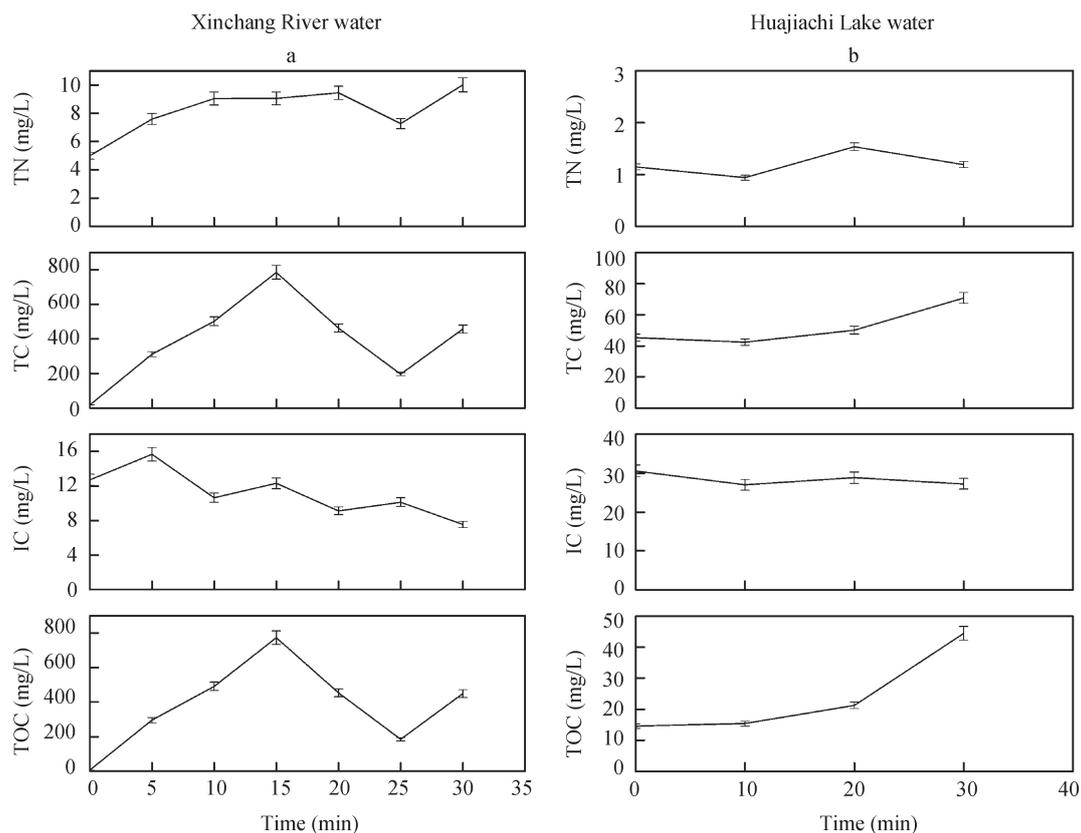


Fig. 3 Variations in carbon and nitrogen levels of water samples after autoclaving in the gradient experiment (5–30 min). (a) water sample collected from Xinchang River (urban river polluted by municipal sewage); (b) water sample collected from Huajiachi Lake (municipal scenic water). The error bars represent the standard deviation ($n = 3$).

the sterilization treatments. These results are similar to that concluded by Leiviskä et al. (2008), who found that the mechanical strain caused by tight ultra-filtration at high pressure converted some of the organic pollutants into more oxidizable forms, which resulted in higher TOC level being measured. Additionally, as shown in Fig. 3, the concentration of IC decreased as the duration of sterilization increased, which was likely due to the escape of IC in the form of CO_2 .

To confirm the alteration of the macromolecular conformation of some organic compounds, the Chl-*a* content in water was determined before and after autoclaving. Chl-*a* is an important indicator of blue-green algae that exists diffusely in reservoirs and lakes. As a photosensitive metalloporphyrin, the ability of Chl-*a* to absorb and transmit light depends on the π - π conjugated molecular structure of the porphyrin cycle (Gouterman, 1978). The concentration of Chl-*a* in water samples from Huajiachi Lake decreased remarkably from $(86.73 \pm 3.62) \mu\text{g/L}$ to $(7.21 \pm 0.08) \mu\text{g/L}$ after autoclaving (30 min). According to the principle of fluorescence quenching measurement for Chl-*a*, the decline in Chl-*a* concentration indicated that the conjugated structures of Chl-*a* were destroyed during the sterilization process.

2.3 Effect of ^{60}Co γ -radiation on TOC

Treatment with ^{60}Co γ -radiation induces the chemoreception or denaturalization of active cellular materials,

Table 3 Variation in TOC, IC and TC before and after ^{60}Co γ -radiation treatment

Parameter	Test time	T_A	$T_{B-^{60}\text{Co}}$	Relative error (%)
TOC (mg/L)	Day 1	7.27 ± 0.71	49.22 ± 0.31	577.00
	Day 3	7.67 ± 0.66	40.94 ± 2.01	433.80
	Day 5	7.06 ± 0.53	42.18 ± 0.97	497.50
IC (mg/L)	Day 1	32.84 ± 0.37	16.80 ± 2.04	-48.80
	Day 3	32.68 ± 1.14	26.04 ± 0.19	-20.30
	Day 5	32.67 ± 0.51	26.03 ± 0.47	-20.30
TC (mg/L)	Day 1	40.11 ± 1.77	66.02 ± 1.98	64.60
	Day 3	40.35 ± 0.78	66.98 ± 0.93	66.00
	Day 5	39.73 ± 0.43	68.21 ± 2.56	71.70

T_A : untreated water; $T_{B-^{60}\text{Co}}$: treated by ^{60}Co γ -radiation.

which results in an injury or death to bacteria. Accordingly, ^{60}Co γ -radiation has been used to sterilize solid and liquid samples. However, the results of our experiment indicated that, although ^{60}Co γ -radiation treatment completely sterilized the water samples, it also induced significant changes in the TOC and TC measurements (Table 3). Specifically, the measured TOC of $T_{B-^{60}\text{Co}}$ (treated by ^{60}Co γ -radiation) increased significantly by 5–7 times when compared with that of the untreated water (T_A), while the measured TC increased by 60%–70% when compared with T_A .

TOC is determined by subtracting the measured TIC from the measured TC value; therefore, magnifying TC or minimizing TIC will lead to an increase in the measured

TOC. However, this does not explain the increasing range of TOC that was observed in the present study. It is possible that ^{60}Co γ -radiation led to the degradation of macromolecular organic material, resulting in the formation of smaller fractions and more oxidizable forms of organic material. Indeed, ^{60}Co γ -radiation stimulates the ionization of atoms or molecules, which can lead to the disruption of excited chemical bonds, resulting in the formation of free radicals or excitation. This process may be the source of the radiation-chemical reaction of organic compounds. Shen et al. (1997) reported that the content of Luteinizing Hormone-Releasing Hormone analogs of post-irradiated injections decreased dramatically in response to ^{60}Co γ -radiation, and that radiation induced a decrease in the intrinsic viscosity of polycaprolactone in response to increased radiation.

As shown in Table 3, the post-irradiated TC was 64.6% greater than that of the pre-irradiation value, and this value continued to increase with time after treatment. Indeed, on the 5th day post-irradiation, the measured TC had increased by 71.7% when compared with the pre-irradiated TC. These results imply that there is a residual disintegrating reaction of the macromolecular compounds in water samples after ^{60}Co γ -radiation treatment.

2.4 Effect of MCUR treatment on TOC

A series of experiments were conducted to evaluate the effect of MCUR on TOC/TC measurements. Water samples were first filtered through 0.45 μm millipore filters, after which they were subjected to 5–70 min of sterilization by ultraviolet radiation at a power and average power density of 40 W and 100–120 $\mu\text{W}/\text{cm}^2$, respectively. The results showed that the MCUR sterilization (> 25 min) had an efficient bactericidal efficacy. Additionally, the measured TOC and other water parameters were unchanged by the MCUR treatments (Fig. 4).

Ultraviolet radiation inhibits the replication of DNA via the induction of thymine dimers. Simultaneously, O_2 and H_2O are able to oxidize into O_3 and H_2O_2 , which subsequently enhance the efficacy of sterilization. Because

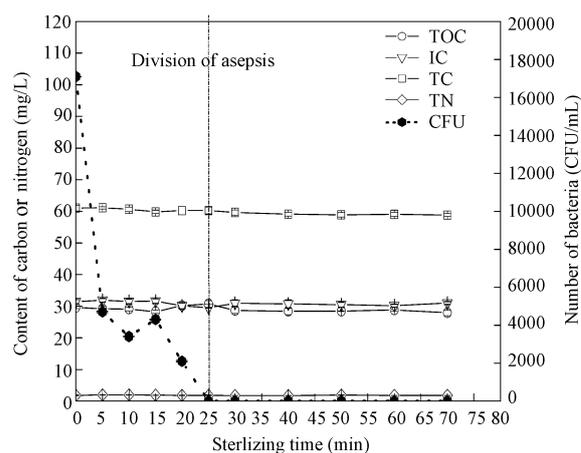


Fig. 4 Effects of sterilization and the variations in carbon and nitrogen levels of wastewater samples sterilized by MCUR in the gradient experiment (5–70 min). Error bars represent the standard deviation ($n = 3$).

only smaller microorganisms can exist after filtration of the water sample, 30 min of ultraviolet radiation is sufficient to sterilize the sample (Fig. 4). Due to the poor ability of ultraviolet radiation to penetrate water, it is very important to maintain a sufficient dosage of ultraviolet radiation and fully expose water samples being treated. Additionally, when sampling water containing organic pollutants, it is important to avoid excessive photolysis, accordingly, radiation gradient experiments should be conducted to determine the optimal radiation dosage, which depends on the bactericidal efficacy of T_B and the relative error of TOC values between T_A and T_B treated by MCUR sterilization.

In the batch experiments, samples with a wide concentration range were selected to assess the effect of the MCUR sterilization on the measured TOC values. The results revealed that MCUR sterilization had little negative effect on the TOC measurements (Table 4). As shown in Table 4, there were small relative differences in the TOC values of T_A and T_B treated by MCUR sterilization, with differences ranging from -1.75% to 2.83% and an average of 0.86% . Additionally, the RSD (RSD = SD/concentration) ranged from 0.4% to 6.5% with

Table 4 Comparisons of TOC, IC, TC and TN before and after MCUR sterilization treatment (30 min)

Sample code	Treatment	Measurement matrix (mg/L, mean \pm SD)			
		TOC	IC	TC	TN
1	T_A	27.02 \pm 1.15	31.71 \pm 0.55	58.72 \pm 0.61	1.71 \pm 0.57
	$T_{B-30\text{min}}$	27.18 \pm 0.56	31.81 \pm 0.79	58.99 \pm 0.23	1.60 \pm 0.23
	Relative errors (%)	0.57	0.33	0.45	-6.14
2	T_A	28.89 \pm 1.26	31.18 \pm 1.31	60.07 \pm 0.06	1.77 \pm 0.01
	$T_{B-30\text{min}}$	28.38 \pm 1.52	30.73 \pm 1.57	59.12 \pm 0.38	1.74 \pm 0.04
	Relative errors (%)	-1.75	-1.42	-1.59	-1.69
3	T_A	35.57 \pm 0.43	32.22 \pm 0.06	67.79 \pm 0.37	1.77 \pm 0.16
	$T_{B-30\text{min}}$	36.47 \pm 2.04	32.72 \pm 1.22	68.70 \pm 0.91	1.79 \pm 0.09
	Relative errors (%)	2.54	0.02	1.34	1.79
4	T_A	132.9 \pm 3.39	44.00 \pm 1.44	176.9 \pm 1.98	3.04 \pm 0.37
	$T_{B-30\text{min}}$	136.8 \pm 2.69	44.90 \pm 0.94	181.7 \pm 1.77	3.08 \pm 0.07
	Relative errors (%)	2.83	1.98	2.64	1.3
5	T_A	159.3 \pm 0.71	43.64 \pm 0.80	202.9 \pm 0.77	2.89 \pm 0.01
	$T_{B-30\text{min}}$	159.5 \pm 0.64	45.80 \pm 2.24	205.2 \pm 1.56	2.85 \pm 0.20
	Relative errors (%)	0.09	4.94	1.18	-1.04
Average relative errors (%)		0.86	1.17	0.8	-1.16
Average relative standard deviation (%)		2.93 \pm 2.13	2.95 \pm 1.57	0.64 \pm 0.40	8.64 \pm 9.92

T_A : untreated water; $T_{B-30\text{min}}$: treated by MCUR (30 min). Sample codes denote the samples with different TOC concentrations in the batch experiments.

an average of 2.93%. These findings suggested that the method of MCUR sterilization has very little effect on the measured TOC in water samples; therefore, this method could improve the stability, adaptability and reliability of sterilization treatment employed to analyze the effects of microbial activity on the degradation and transformation of organic pollutants in water.

3 Conclusions

Because organic compounds are sensitive to drastic sterilization processes, the pyrolysis caused by autoclaving can have a negative effect on the measurement of TOC, thereby resulting in the TOC of autoclaved water samples being significantly different from that of untreated samples. In the present study, the variation in measured TOC values between pre-sterilized and post-sterilized treatments was found to vary greatly among categories and concentrations of organic pollutants. Sterilization using ^{60}Co γ -radiation led to a significant increase in the measured values of TOC and TC in water samples via a disintegrating reaction with the macromolecules. In addition, a residual disintegrating reaction in response to ^{60}Co γ -radiation was observed.

The MCUR sterilization method, which consisted of 0.45 μm micro-filtration coupled with ultraviolet radiation of the filtered water sample, had a high efficiency of sterilization and little effect on the organic compounds in the water samples. Before and after sterilization treatments, the relative errors of TOC were less than 3% for water samples containing a variety of categories and concentrations of organic pollutants. These results suggested that the MCUR sterilization method was relatively simple and effective, especially with respect in maintaining the measured TOC values between sterilized and non-sterilized water samples.

Acknowledgments

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