

An improved method for isotopic and quantitative analysis of dissolved organic carbon in natural water samples

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ABSTRACT

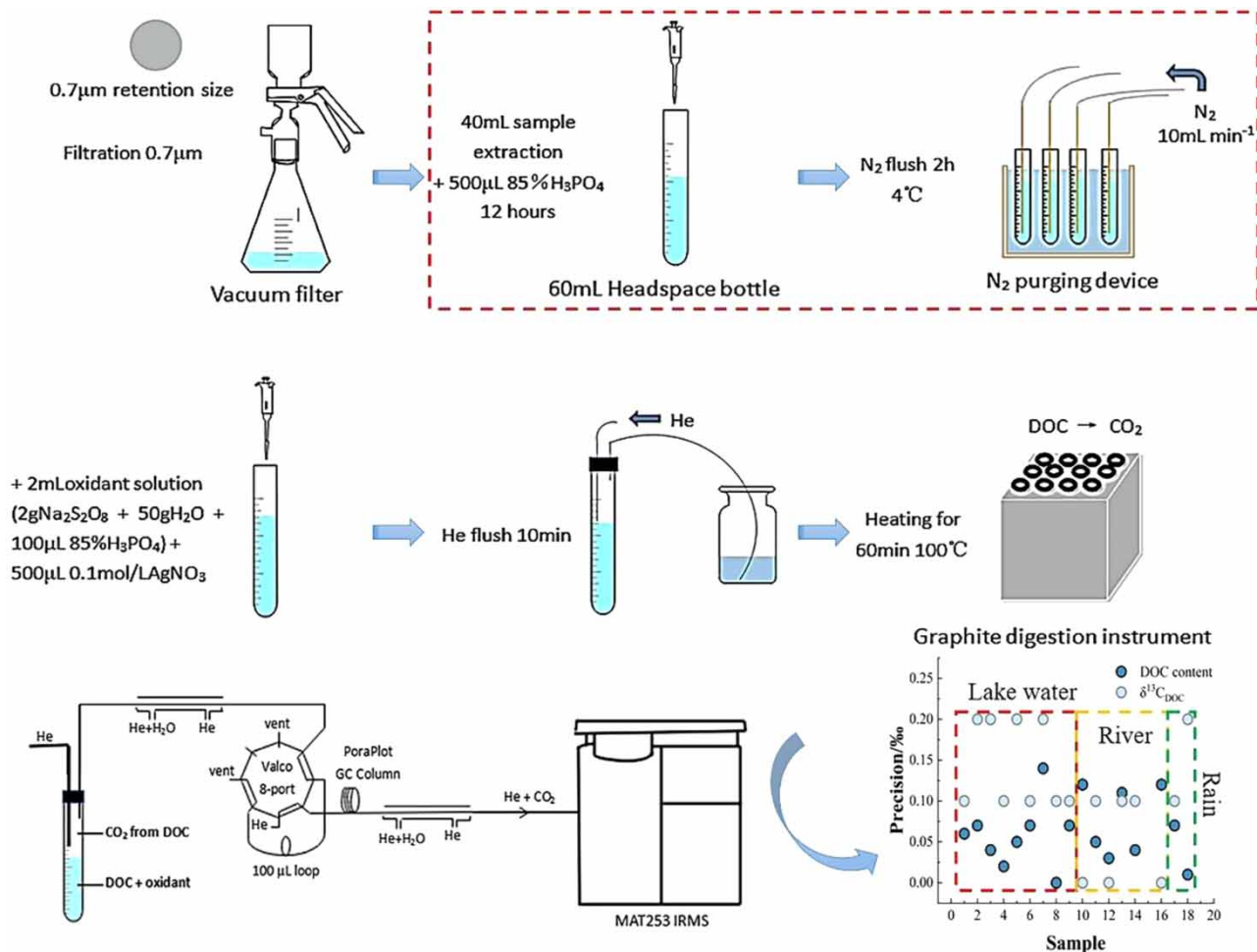
A wet chemical oxidation (WCO) method has been widely used to obtain the dissolved organic carbon (DOC) content and carbon isotope ($\delta^{13}\text{C}_{\text{DOC}}$) ratios. However, it is sometimes difficult to get high precision results because not enough CO_2 was oxidized from the natural water samples with low DOC concentrations. This improvement primarily aims to increase the water sample volume, improve the removal rate of dissolved inorganic carbon (DIC), and minimize the blank DOC from the standard solution. Following the improved procedure, the $\delta^{13}\text{C}$ ratios of standardized DOC solutions were consistent with their actual values, and their differences were less than 0.2‰. The improved method demonstrated good accuracy and stability when applied to natural water samples with DOC concentrations $\geq 0.5 \text{ mg L}^{-1}$, with the precisions of DOC concentrations and $\delta^{13}\text{C}$ ratios were better than 0.07 mg L^{-1} and 0.1‰, respectively. More importantly, this method saved much pre-treatment time and realized batch processing of water samples to obtain their DOC contents and isotope ratios.

Key words: aquatic environment, carbon isotope ratios, dissolved inorganic carbon removal, dissolved organic carbon, wet oxidation method

HIGHLIGHTS

- The CO_2 peak values of the water samples were within the optimal testing range of the instrument.
- Low-temperature nitrogen purging can more thoroughly remove DIC from water samples.
- The precision of the DOC concentration in the water samples was better than 0.07 mg L^{-1} .
- The precision of the $\delta^{13}\text{C}_{\text{DOC}}$ values in the water samples was better than 0.1‰.

GRAPHICAL ABSTRACT



1. INTRODUCTION

Dissolved organic carbon (DOC) is the major form of organic carbon (OC) exported from the watershed (David *et al.* 1992), and the flux of DOC transported to the ocean via the river system was about 200 Mt C yr $^{-1}$ (Kirschbaum *et al.* 2019). DOC is closely related to the acid–base chemistry of acid-sensitive freshwater systems, as well as mobility, persistence, and toxicity of metals and other pollutants (David *et al.* 1992). DOC carbon isotope ($\delta^{13}\text{C}_{\text{DOC}}$) has been widely used to track DOC sources, transport, and fate in the watershed (David *et al.* 1992). The measurement of DOC content and $\delta^{13}\text{C}_{\text{DOC}}$ ratios were helpful to solve these problems and identify the impact of human activities in the aquatic environment (Zhang *et al.* 2020b; Li *et al.* 2024). Hence, accurate determination of DOC content and $\delta^{13}\text{C}_{\text{DOC}}$ ratios were essential to satisfy these demands. Previous studies have conducted much research on the pre-treatment method for the above parameters to obtain stable and believable results (Keys 1934; Sharp 1973; Romankevich 1984; Skrzypek 2013).

High-temperature combustion (HTC) (Lang *et al.* 2007; Federherr *et al.* 2014) and wet chemical oxidation (WCO) (Osburn & St-Jean 2007; Zhou *et al.* 2015) were two predominate pre-treatment methods for determining DOC content and $\delta^{13}\text{C}_{\text{DOC}}$ ratios. These methods involve oxidizing DOC to carbon dioxide (CO_2) (Federherr *et al.* 2014), which is then introduced into a stable isotope ratio mass spectrometer (IRMS) with helium to analyze the m/z 44 signal value and isotopic composition of CO_2 (Gandhi *et al.* 2004). The primary difference between these two methods is the oxidative pathways of DOC (Romankevich 1984; Lang *et al.* 2012). DOC was transferred to CO_2 via a catalyst and high-temperature furnace combustion in the HTC method (Skopintsev *et al.* 1966; Romankevich 1984; Federherr *et al.* 2014), but through chemical oxidants (K_2O_2 , H_2O_2 , KMnO_4 , $\text{K}_2\text{Cr}_2\text{O}_7$, $\text{K}_2\text{S}_2\text{O}_8$, and $\text{Na}_2\text{S}_2\text{O}_8$) in the WCO method (Menzel & Vaccaro 1964; Skopintsev 1976; Mckenna

& Doering 1995; Yu *et al.* 2015; Skrzypek & Ford 2019). While the WCO method may not achieve complete oxidation compared to HTC, it significantly saves time by eliminating laborious steps like freeze-drying. Moreover, its results are stable, and contamination and blank issues are controllable (Romankevich 1984; Lang *et al.* 2012). The persulfate oxidant has been established as the standard method for seawater DOC determination by Menzel and Wilson (Wilson 1961; Menzel & Vaccaro 1964). Later, Zhou developed the oxidation conditions of the persulfate wet oxidation method (Zhou *et al.* 2015) and found that the precision using $\text{Na}_2\text{S}_2\text{O}_8$ as persulfate oxidant was better than that using $\text{K}_2\text{S}_2\text{O}_8$ oxidant (Zhou *et al.* 2015). Meanwhile, the incorporation of silver nitrate catalyst and heating in a water bath at 100 °C for 1 h facilitated the full oxidation of OC (Zhou *et al.* 2015).

In addition, dissolved inorganic carbon (DIC) must be eliminated before the oxidation of DOC because the CO_2 produced by DIC will interfere with the DOC content and $\delta^{13}\text{C}_{\text{DOC}}$ ratios (Yu *et al.* 2015; Skrzypek & Ford 2019). Several DIC removal methods have been developed and modified previously (Assayag *et al.* 2006; Yu *et al.* 2015). The conventional techniques included the direct precipitation of DIC into carbonates and evolved gas for acidifying DIC with phosphoric acid (Assayag *et al.* 2006; Zhou *et al.* 2015; Yu *et al.* 2015). Previous studies have demonstrated that the DIC removal is not complete with such methods (Lang *et al.* 2012). The CO_2 produced by the DIC of the sample is partitioned between the gaseous and dissolved phases in the vials and a carbon isotope fractionation between gaseous and dissolved CO_2 results from this partition (Lang *et al.* 2012). For example, Zhou *et al.* found that the testing accuracy of solid standard samples when establishing the method was better than 0.2‰, but once the method was applied to groundwater, the precision decreased to ~0.35‰ (Zhou *et al.* 2015). Therefore, it is necessary to keep the same circumstance and procedure for substance used to compare impacts from variable factors on DOC contents and isotope measurement (Rock & Mayer 2006). This defect reminded us that dissolved solid reference would produce blank error from ultrapure water. Hence, if more water was pre-treated to elevate the CO_2 signals, the blank error due to the large volume of ultrapure water input should be considered. Therefore, it is necessary to investigate the effect of water volume and DIC removal effects on the (m/z 44) signals of the CO_2 generated by DOC.

In this study, we tried to increase the water volume to produce enough CO_2 to obtain better precision and accuracy of measurement by Gasbench II-IRMS. To remove the internal DIC, we used the nitrogen purging process under the ice bath. Organic and inorganic carbon solids with known $\delta^{13}\text{C}$ values were dissolved into ultrapure water as DOC, DIC, and mixed standard solutions. These standard solutions were oxidized, and their measured $\delta^{13}\text{C}$ values were compared with their real $\delta^{13}\text{C}$ values to check the effectiveness of our improvements.

2. EXPERIMENTAL

2.1. Preparation of solid laboratory references

Recent research has demonstrated that incomplete oxidation of samples may cause isotope fractionations during WCO (Skrzypek & Ford 2019). To constrain this disturbance, four solid reagents were chosen as the laboratory reference substances for DOC, which included potassium hydrogen phthalate (KHP) and acetylaniline (ACE), sucrose (SUC), and L(+)-glutamic acid (GLU) (guaranteed reagent, GR, Shanghai Aladdin Biochemical Technology Co., Ltd). Their $\delta^{13}\text{C}$ values were measured using an Elemental Analyzer (EA, Flash 2000 HT, Thermo) coupled with a MAT-253 IRMS (Thermo Fisher-Scientific, Bremen, Germany) (Zhang *et al.* 2020a) (Table 1), and international standard substance IAEA-600 ($\delta^{13}\text{C} = -27.771\text{‰}$) was used to calibrate their isotope values.

Table 1 | The selected DOC and DIC references and their $\delta^{13}\text{C}$ ratios

Compound	Formula	Molecular weight g/mol	Wt. %	CAS	Solubility g/100water	Measured $\delta^{13}\text{C}$ ‰
KHP	$\text{C}_8\text{H}_5\text{O}_4\text{K}$	204.22	99.99	877-24-7	80/20 °C	-29.1 ± 0.2
ACE	$\text{C}_8\text{H}_9\text{NO}$	135.17	99.00	103-84-4	0.56/25 °C	-28.0 ± 0.2
SUC	$\text{C}_{12}\text{H}_{22}\text{O}_{11}$	342.3	≥ 99.00	57-50-1	2,000/25 °C	-12.9 ± 0.2
L(+)-GLU	$\text{L}(+)\text{-C}_5\text{H}_9\text{NO}_4$	147.13	≥ 99.50	56-86-0	0.72/20 °C	-12.7 ± 0.2
Sodium bicarbonate	NaHCO_3	84.007	≥ 99.80	144-55-8	7.8/18 °C	-10.5 ± 0.1

In the aquatic environment, bicarbonate (HCO_3^-) was the dominant component of DIC in the stream water (Das *et al.* 2005; Barnes & Raymond 2009). Therefore, NaHCO_3 (guaranteed reagent, GR, Shanghai Aladdin Biochemical Technology Co., Ltd) was selected as the DIC standard material in the laboratory. The $\delta^{13}\text{C}$ ratios of solid NaHCO_3 were obtained by the traditional phosphoric acid method (Krishnamurthy & Atekwana 1997). Four to six drops of phosphoric acid (density 1.91) were injected into a 12 mL Exetainer tube (Labco, High Wycombe, UK) where solid NaHCO_3 (about 100 μg) was installed and purged by helium gas (99.999%) in advance. The headspace CO_2 was transported and analyzed using MAT 253 equipped with an online Gasbench II interface (Du & Song 2020). International standard carbonate solid NBS 18 ($\delta^{13}\text{C} = -5.014\text{‰}$) was used to calibrate the $\delta^{13}\text{C}$ ratios of our laboratory reference. The $\delta^{13}\text{C}$ ratios of solutions were also determined by the phosphoric acid method (Spötl 2005). About 0.5 mL of phosphoric acid (85% (V/V)) was injected into a 12-mL Exetainer tube (Labco, High Wycombe, UK) and purged with Helium gas (99.999%) to remove residual CO_2 . Then, a syringe injected about 0.5–2 mL solutions into the 12-mL Exetainer tube. The headspace CO_2 was transported and analyzed through MAT 253 coupled with an online Gasbench II interface (Du & Song 2020). There is no obvious alternation of $\delta^{13}\text{C}$ ratios between solid NaHCO_3 and its solutions due to no DIC in the ultrapure water.

2.2. Preparation of DOC and DIC reference solutions

2.2.1. DOC blank in ultrapure water

To eliminate the background DOC interference in ultrapure water, we chose four types of ultrapure water and compared their DOC contents followed by our established procedure. These four types of ultrapure water included commercial bottle water with the brand Wahaha, C'estbon, Watson, and our laboratory ultrapure water (Supplementary material, Table S1). No solid reference substance was added to these ultrapure water samples, and the CO_2 peaks and DOC contents from these ultrapure water samples were listed in Supplementary material, Table S1.

2.2.2. DOC reference solutions for DOC concentration determination

To obtain DOC concentrations of water samples tested, a relation between the known DOC concentration and produced CO_2 amount must be established. KHP was used as a DOC reference because it was not easy to oxidize by WCO and has high solubility (Table 1). If a good linear relationship could be obtained with KHP solutions, other similarly oxidized organic substances could also be used to establish this similar linear relationship. Three DOC concentration gradients of 3, 6, and 9 mg L^{-1} , respectively, with four water volumes of 10, 20, 30, and 40 mL, were made to establish the above relations (Supplementary material, Table S2).

2.2.3. DIC reference solutions for DIC removal rate

DIC reference solutions were prepared using NaHCO_3 to limit the effect of different DIC removal methods on the final $\delta^{13}\text{C}$ value (Supplementary material, Table S3). In addition, to verify the feasibility of the improved DIC removal method, DIC/DOC mixed standard solutions were also prepared using NaHCO_3 and KHP (Supplementary material, Table S3).

2.3. The established pre-treatment procedure

2.3.1. Pre-treatment of glassware

The glassware used in the experiment was soaked with 1 mol L^{-1} of sodium hydroxide (NaOH , GR, 99.9%, CAS: 1310-73-2, Aladdin reagent) overnight and was cleaned by ultrapure water, and then calcined at 500 °C in the Muffle furnace for 5 h to remove organic matter completely.

2.3.2. Methods of acidification and oxidation

The reference solutions in Supplementary material, Table S2 were added 500 μL of 85% H_3PO_4 , then shaken and still for 12 h. Two milliliters of oxidant (50 mL $\text{H}_2\text{O} + 2 \text{ g Na}_2\text{S}_2\text{O}_8 + 100 \mu\text{L}$ 85% H_3PO_4) and 100 μL catalyst made of 0.1 mol L^{-1} AgNO_3 ($\geq 99.8\%$, CAS: 7761-88-8, Sinopharm Chemical Reagent Co. Ltd) were added into the liquids (Zhou *et al.* 2015). High-purity helium of 99.999% was used to remove residual CO_2 at 20 mL min^{-1} for 10 min. Ultimately, these bottles were heated on a graphite furnace under 100 °C for 1 h and then cooled to room temperature (Sharp 1973).

2.3.3. Verification of DIC removal

After acidification, the dissolved CO_2 in solutions should be removed to eliminate their disturbance on final isotope ratios. This study did not include a control experiment to investigate the necessity of ice-bath conditions during the DIC purging

process. Following previous research findings, lower temperatures are more conducive to the recovery of volatile organic carbon (VOCs) (Xu *et al.* 2022). Therefore, in this study, high-purity nitrogen gas (99.999%) was utilized at 4 °C in an ice bath to remove dissolved CO₂. The DIC and DOC/DIC mixed reference solutions were utilized to test the effectiveness of the DIC removal method (Supplementary material, Table S3).

2.4. Preparation of natural water samples

River water, lake water, and rainwater were used to test our improved method. These samples were filtered using a pre-burned Whatman GF/F filter (burning at 450 °C for 5 h) with a porosity of 0.7 mm and stored in a pre-treated glass bottle (Ge *et al.* 2020). All samples were added with two drops of saturated HgCl₂ (Kang *et al.* 2019) and kept in the dark environment at ≤4 °C to limit microbial activity. The improved method pre-treated the natural water samples to obtain their DOC contents and δ¹³C_{DOC} ratios.

2.5. Determination of DOC content and δ¹³C values

The DOC content was obtained by the linear relationship between DOC amount (μg) and the CO₂ peak (*m/z* 44, mV) of standard KHP solutions. The oxidized CO₂ was carried by Helium gas and reloaded to the MAT 253 through a GasBench II peripheral equipment.

The δ¹³C value is defined as (Wen *et al.* 2020):

$$\delta^{13}\text{C}(\text{‰}) = 1000 \times [(R_{\text{sample}}/R_{\text{standard}}) - 1] \quad (1)$$

where R_{sample} and R_{standard} are the ratios of ¹³C to ¹²C in the sample and the international standard Vienna Pee Dee Belemnite (VPDB), respectively, and it was worth noting that the δ¹³C_{DOC} ratios of KHP were calculated based on the δ¹³C values of CO₂ reference gas due to no DOC reference solutions with known δ¹³C_{DOC} ratios in the market.

2.5.1. Blank correction

Blank effects of DOC concentrations and δ¹³C_{DOC} values were evaluated using CO₂ peaks (M) and isotopes. The peak area obtained from the measurement can represent the carbon content in the tested sample/standard. The program blank can be corrected according to the following quality balance.

$$\delta^{13}\text{C}_{\text{meas}} \times M_{\text{meas}} = \delta^{13}\text{C}_{\text{sample}} \times M_{\text{sample}} + \delta^{13}\text{C}_{\text{blank}} \times M_{\text{blank}} \quad (2)$$

where δ¹³C_{meas}, δ¹³C_{sample} and δ¹³C_{blank} are the measured δ¹³C, the δ¹³C of samples and the δ¹³C of procedural blanks, respectively. M_{meas} and M_{blank} denote the peak areas of the measurements and blanks, correspondingly.

In order to calibrate the contribution of program blanks to isotope results, the CO₂ peaks and δ¹³C_{blank} of blank samples were calculated by indirect method, and compared with the peak and δ¹³C values of blank samples determined directly. KHP and SUC at different concentrations were determined to calculate M_{blank} and δ¹³C_{blank}. Its isotope range is wide, and it can basically cover δ¹³C_{DOC} of most natural water samples.

The linear relationship between δ¹³C values and CO₂ peaks a reciprocal (1/ M) is observed from Equation (2). According to the keeling plot theory, two standards linear equations of δ¹³C values and CO₂ peaks reciprocal are established respectively. The slope is expressed as k_{KHP} , k_{SUC} and the intercept is b_{KHP} , b_{SUC} .

Therefore, the following equation can be used to calibrate the blank effect (Zhang *et al.* 2019):

$$\delta^{13}\text{C}_{\text{sample}} = (\delta^{13}\text{C}_{\text{meas}} \times M_{\text{meas}} - \delta^{13}\text{C}_{\text{blank}} \times M_{\text{blank}})/(M_{\text{meas}} - M_{\text{blank}}) \quad (3)$$

3. RESULTS AND DISCUSSION

3.1. Ultrapure water with low DOC background

Because ultrapure water had minor DIC contents, we directly oxidized them with the oxidant and catalyst after phosphoric acid acidification (part 2.3). From Supplementary material, Table S1, it was observed that distilled water yielded the lowest CO₂ peak values after the WCO process, indicating that DOC could be effectively removed through distillation. Therefore, in the subsequent experiments, Watson distilled water was used to address these solid references and obtain standard DOC

solutions. Additionally, DOC blank samples prepared without any substances were also used to quantify the impacts of this distilled water. After reducing these distilled water effects, the DOC content and isotope ratios of reference materials and natural water samples were precisely obtained.

3.2. The optimal volume of water samples

For natural water samples with low DOC content, increased water volume would bring much DOC oxidized and get good precisions for DOC contents and $\delta^{13}\text{C}_{\text{DOC}}$ ratios due to enough CO_2 to be tested. After the WCO, the KHP solutions in Supplementary material, Table S2 created a good linear relationship between DOC amount and CO_2 peak (m/z 44, mV) (Figure 1(a)). This fact tells us that the WCO method can oxidize KHP fully. The isotope values of the last two points fall within the range of true values (Figure 1(b)), indicating that errors in the pre-treatment process can be ruled out. Therefore, the anomalies exhibited by these two points are likely due to oversights during sample weighing (Figure 1(a)), and we have placed particular attention on this phenomenon in subsequent experimental procedures. The obtained $\delta^{13}\text{C}_{\text{DOC}}$ ratios of KHP in different volumes of solutions were variable (Figure 1(b)). Only KHP solutions with 30 and 40 mL had similar $\delta^{13}\text{C}_{\text{DOC}}$ ratios to that measured by EA-IRMS ($\delta^{13}\text{C} = -29.1 \pm 0.2\text{‰}$). DOC ranging from approximately 60–160 μg can yield optimal CO_2 peaks. Therefore, selecting a water volume of 40 mL ensures that the DOC concentration ranging from 1.5 to 4 mg L^{-1} falls within the optimal testing range, meeting the testing requirements of the majority of natural water samples.

In addition, studies have shown that isotope fractionation occurs between gaseous and dissolved CO_2 in headspace bottles at room temperature (fractionation coefficient $\varepsilon_{\text{ag-g}} = -1.2 \pm 0.06\text{‰}$) (Zhang *et al.* 1995). The relationship between the real carbon isotope values of CO_2 produced by wet oxidation water samples in the vial and the $\delta^{13}\text{C}_{\text{CO}_2}$ values in the headspace bottle satisfies the following equation (Skrzypek *et al.* 2010; Lang *et al.* 2012):

$$F_H = 1/[(V_L \times R \times T)/V_H + 1] \quad (4)$$

$$\delta^{13}\text{C}_{\text{total}} = (1 - F_H) \times \delta^{13}\text{C}_H + F_H \times (\delta^{13}\text{C}_H - \varepsilon_{\text{ag-g}}) \quad (5)$$

where $\delta^{13}\text{C}_{\text{total}}$ is the $\delta^{13}\text{C}$ value of total CO_2 produced by oxidation of the solution in the headspace bottle, V_L is the volume of liquid in the vial, and V_H is the volume of the headspace in the vial. Moreover, $\delta^{13}\text{C}_H$ is the $\delta^{13}\text{C}_{\text{CO}_2}$ value of the headspace CO_2 in the vial. The relationship between the true carbon isotope values of samples and the carbon isotope values of the headspace CO_2 in the vial by re-arranging these two equations:

$$\delta^{13}\text{C}_{\text{total}} = \delta^{13}\text{C}_H - [(V_H + 1)/(V_L \times R \times T)] \times \varepsilon_{\text{ag-g}} \quad (6)$$

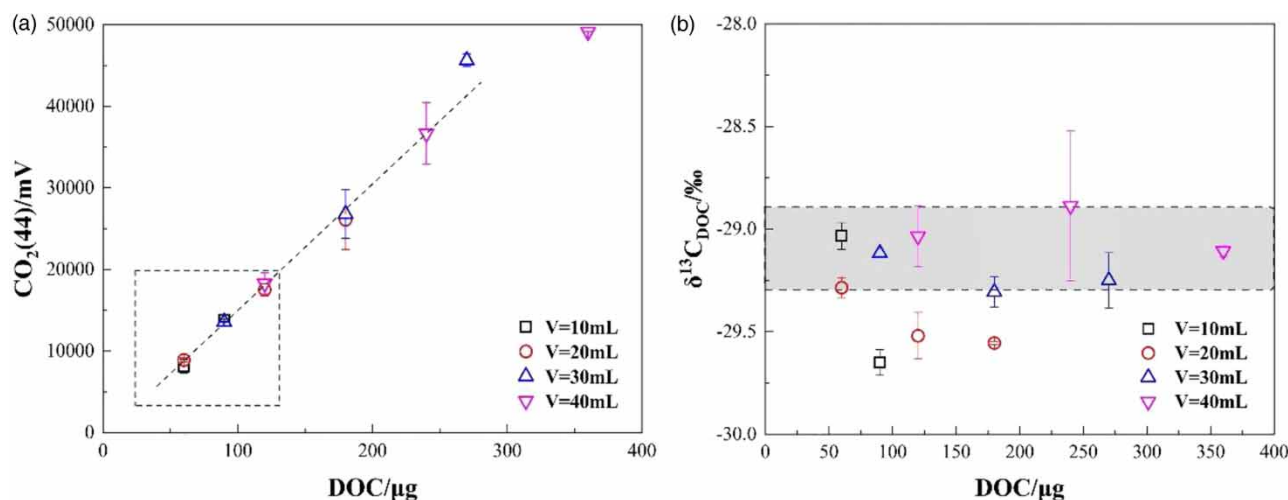


Figure 1 | The CO_2 m/z 44 (a) and $\delta^{13}\text{C}_{\text{GB}}$ values (b) of potassium hydrogen phthalate solutions with different DOC contents (3, 6, and 9 mg L^{-1}) and variable volumes (10, 20, 30, and 40 mL) were compared. The shaded part is the deviation range of $\delta^{13}\text{C}_{\text{EA}}$ values produced by solid potassium hydrogen phthalate.

It can be seen from Equation (4) that the smaller the volume of the upper space, the more the $\delta^{13}\text{C}_H$ value in the headspace bottle can represent the real carbon isotope value of the sample. When the volume of solution in the 60 mL headspace bottle is 30 mL or 40 mL, the peak value can be achieved, but the carbon isotope values are closer to the real values. Besides, the consistency of sample preparation was ensured to follow the same treatment and analysis principles as natural water samples. The improved method is better applied for the pre-treatment of DOC content and isotopic value determination of water samples than the method established to utilize solid reference materials. The final volume of wet oxidation water samples can be determined according to natural water with different DOC concentration ranges. In the subsequent experiments, the focus was primarily on the 40 mL water volume.

3.3. DIC removal rate

Traditional DIC removal by H_3PO_4 was considered in our experiment (Bao *et al.* 2014; Yi *et al.* 2021). The NaHCO_3 solutions of the volume of 40 mL with different concentrations were added with 500 μL H_3PO_4 (85 wt.%) and stilling for 12 h. After that, high-purity nitrogen gas was used to purge the DIC solutions at 10 mL min^{-1} for 1 and 2 h in an ice bath under 4 °C, respectively. As illustrated in Figure 2, the mean $\delta^{13}\text{C}_{\text{DOC}}$ ratio of distilled water samples devoid of DIC was approximately $-25.0 \pm 0.3\text{‰}$ following a 2 h purging period. For DIC reference solutions only purged for 1 h, the $\delta^{13}\text{C}$ ratios were significantly higher than that in the distilled water sample due to incomplete removal of DIC with positive $\delta^{13}\text{C}$ ratios. However, after purging for 2 h, the average $\delta^{13}\text{C}_{\text{CO}_2}$ value of DIC reference solutions was close to that in the distilled water samples. This result told us that after 2 h purging with high-purity nitrogen gas, the dissolved CO_2 due to DIC acidification by H_3PO_4 could be excluded completely.

The removal effect of DIC in the solutions was also tested through the mixed solution of KHP and sodium bicarbonate. If the DIC were completely removed, the $\delta^{13}\text{C}$ ratios of residual DOC would be close to their $\delta^{13}\text{C}$ values determined by EA-IRMS (Figure 3(b)). The CO_2 (m/z 44) peak values obtained by the improved method had a good linear relationship with DOC content ($R^2 = 0.9938$) (Figure 3(a)). Furthermore, the random difference between the $\delta^{13}\text{C}_{\text{GB}}$ values of the mixed solutions and the $\delta^{13}\text{C}_{\text{EA}}$ value of KHP was better than 0.2‰ (Figure 3(b)). The reproducibility of $\delta^{13}\text{C}_{\text{KHP}}$ values did not exceed 0.2‰ (Supplementary material, Table S4).

This result is similar to the reproducibility ($<0.21\text{‰}$) of the $\delta^{13}\text{C}$ values obtained by liquid chromatography-IRMS for humic acid samples in the mixed solution (Federherr *et al.* 2014). In comparison to the pre-treatment method of freeze-drying used by Federherr *et al.*, our pre-treatment method offers advantages such as controlled blanks, cost-effectiveness, and time savings (Federherr *et al.* 2014). Additionally, the precision achieved in this experiment was not only lower than the research results of humic acid samples using liquid chromatography-IRMS ($<0.35\text{‰}$) (Skrzypek & Ford 2019), but

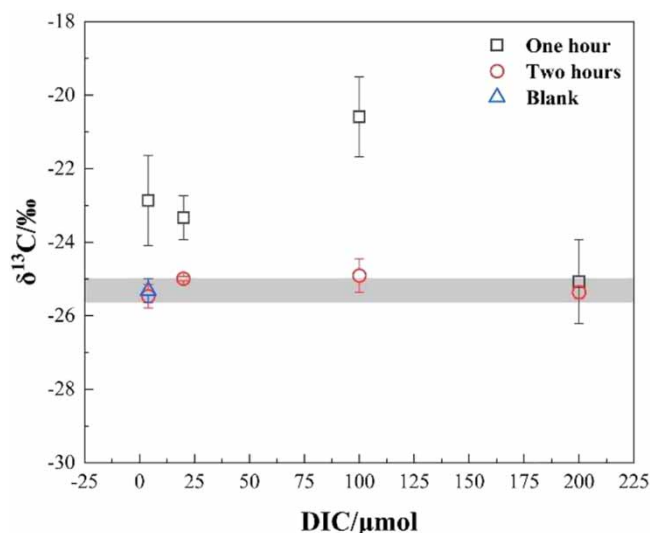


Figure 2 | The $\delta^{13}\text{C}_{\text{GB}}$ values (‰) of DIC reference solutions with nitrogen purge for 1 and 2 h in an ice bath under 4 °C (a). The shaded part is the $\delta^{13}\text{C}$ value obtained from the distilled water samples without DIC after purging for 2 h. After purging for 2 h, the average $\delta^{13}\text{C}_{\text{CO}_2}$ value of solutions was close to that in the distilled water samples.

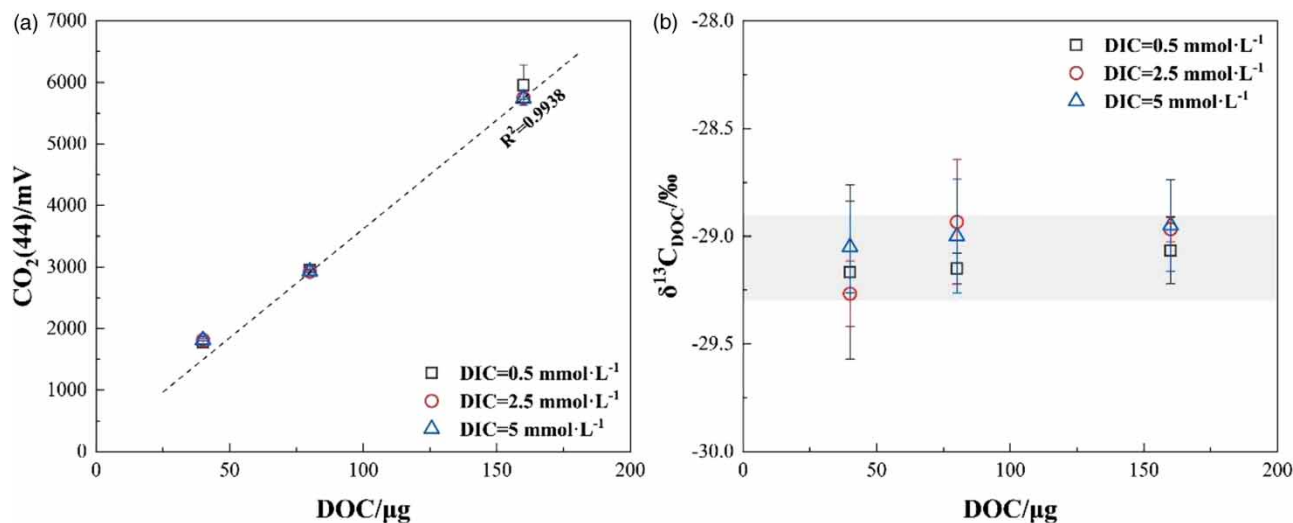


Figure 3 | The CO₂ *m/z* 44 values (a) and δ¹³C_{CO2} (b) of the mixed solutions of KHP and sodium bicarbonate were obtained by the optimized method. The shaded part is the deviation range of δ¹³C_{EA} values produced by solid potassium hydrogen phthalate. The δ¹³C ratios of residual DOC closed to their δ¹³C values determined by EA-IRMS (b). The CO₂ (*m/z* 44) peak values had a good linear relationship with DOC content.

also better than the test results of phthalic acid samples using GasBench-IRMS (0.4‰) (Lang *et al.* 2012), indicating that the improved method is more effective to remove the extra DIC.

3.4. Verification of the improved method

The other three DOC references were also pre-treated with our improved method. The linear relationship between DOC content and the CO₂ *m/z* 44 signal (Figure 4(a)) indicated that our improved method was also suitable for other DOC references when we wanted to obtain the precise DOC contents via the WCO. The CO₂ (*m/z* 44) peak values obtained had a good linear relationship with DOC contents ($R_{\text{SUC}}^2 = 0.9896$, $R_{\text{ACE}}^2 = 0.9996$, $R_{\text{KHP}}^2 = 0.9940$, $R_{\text{L(+)-GLU}}^2 = 0.9996$). The maximum differences between the measured δ¹³C_{DOC} and their actual δ¹³C_{EA} ratios were 0.4‰, 0.6‰, 0.2‰, and 1.3‰, respectively (Figure 4(b)). The corresponding standard deviations were <0.3‰, <0.2‰, <0.1‰, and <0.2‰, respectively.

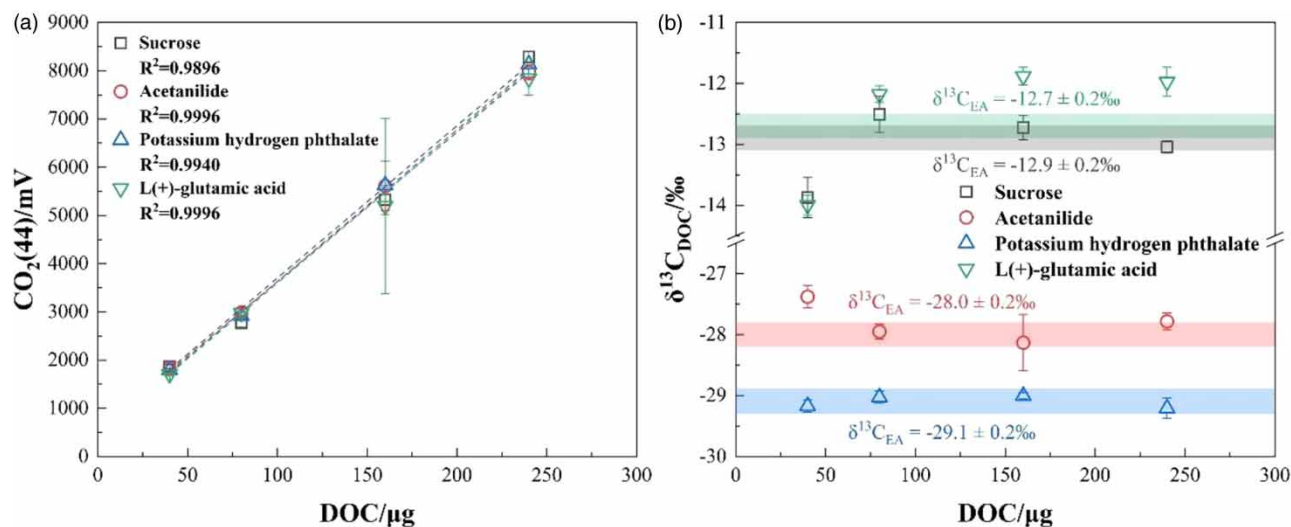


Figure 4 | CO₂ peak values (a) and δ¹³C_{GB} values (b) of the four reference materials vary with carbon content. For this analysis, reference solutions had the same concentration of carbon (1–6 mg L⁻¹). The shaded parts in figure (b) represent their δ¹³C_{EA} values.

The parallelisms of $\delta^{13}\text{C}$ values from the four reference samples were good, and no obvious memory effects were observed. It proves that the mutual interference between samples could be ignored in the GasBench-IRMS test. The significant error observed in the $160\ \mu\text{g L}^{(+)}\text{-GLU}$ measurements may be attributed to gas leakage in the headspace vial during the pre-treatment process (Figure 4(a)), resulting in isotopic enrichment (Figure 4(b)). Furthermore, compounds containing more nitrogen, such as ACE and L(+)-GLU, substantially differed between their $\delta^{13}\text{C}_{\text{GB}}$ values and $\delta^{13}\text{C}_{\text{EA}}$ values (Figure 4(b)). These differences probably arose from the conversion of nitrogen in the compound, which led to stable isotope fractionation (Skrzypek & Ford 2019). This phenomenon could potentially serve as a limitation of the present methodology.

3.5. Pre-treatment procedures and calculations for DOC concentration and isotopic values

3.5.1. The procedure established for the determination of DOC contents and $\delta^{13}\text{C}_{\text{DOC}}$ values

After solving the disturbances from DIC and DOC blank, we established our procedure to determine DOC contents and $\delta^{13}\text{C}_{\text{DOC}}$ values (Figure 5). First, the glass used in the experiment was pre-combusted in a muffle furnace at $500\ ^\circ\text{C}$ for 5 h to remove organic matter. The second step was DIC removal, and 40 mL water samples were added into 60 mL headspace vials. The solutions were added $500\ \mu\text{L}$ of H_3PO_4 (85 wt. %), and the liquids in the vials were shaken and still for 12 h. Then the vials were opened and purged/bubbled for 2 h using nitrogen with an ultra-high-purity of 99.999% in an ice bath under $4\ ^\circ\text{C}$ (flow rate: $10\ \text{mL min}^{-1}$; Needle: made of silica capillary). Following the purification steps, 2 mL oxidant ($2\ \text{g Na}_2\text{S}_2\text{O}_8 + 50\ \text{g H}_2\text{O} + 100\ \mu\text{L 85\% H}_3\text{PO}_4$) and $100\ \mu\text{L}$ catalyst ($0.1\ \text{mol L}^{-1}\ \text{AgNO}_3$ solution) agents were added into the samples (Zhou *et al.* 2015). The nest bottles were sealed up and purged with ultra-high-purity helium (99.999%, 20 mL

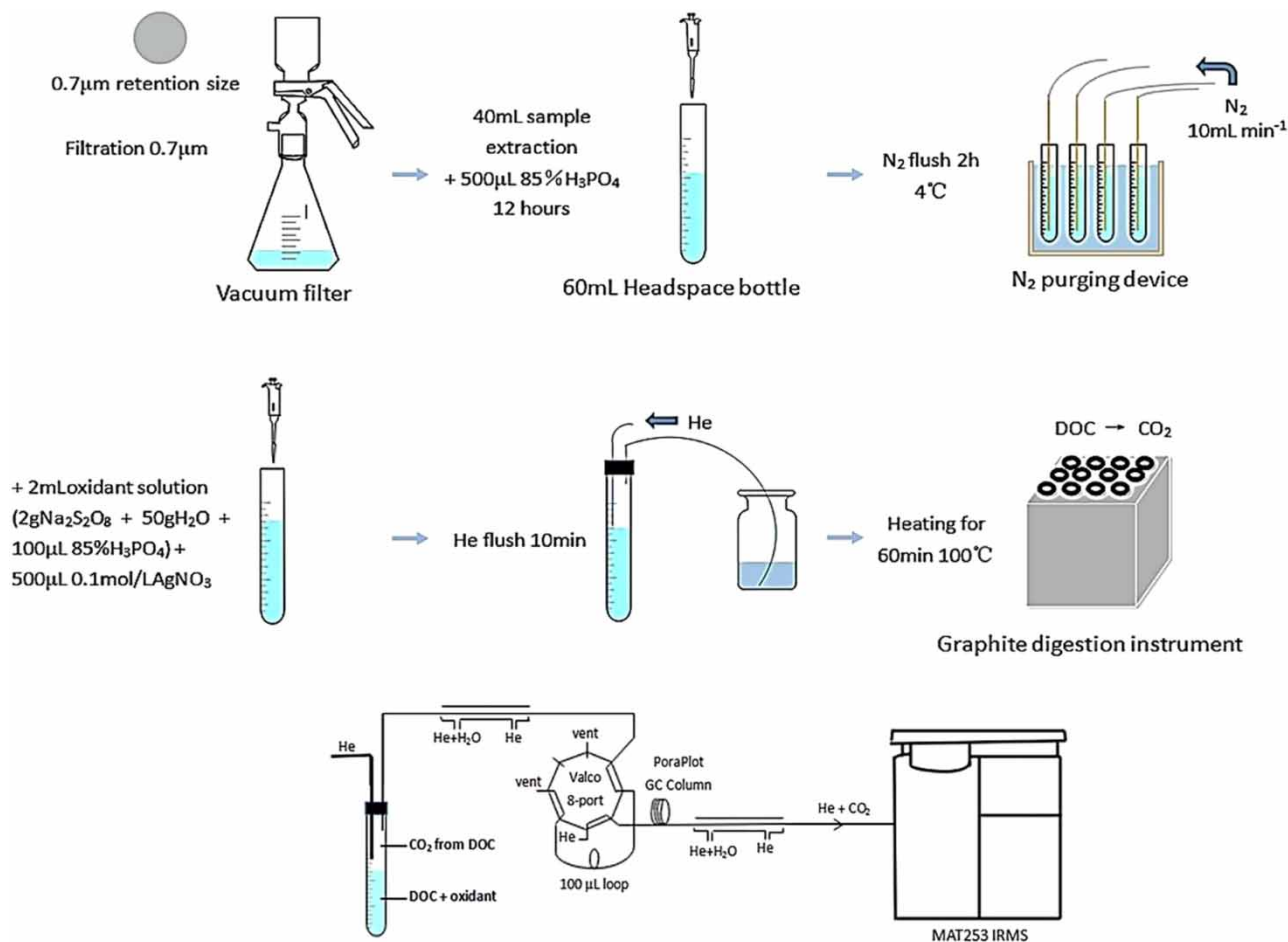


Figure 5 | The established procedure for DOC content and $\delta^{13}\text{C}$ value determination. Schematic showing the various steps involved in the improved pre-treatment conditions and the GasBench-IRMS injection procedure used in our laboratory.

min^{-1}) for 10 min. The sealed headspace bottles were heated on a graphite furnace controlled at 100 °C for 60 min and cooled to room temperature.

Ultimately, the m/z 44 signal values and $\delta^{13}\text{C}$ values produced by CO_2 in the headspace bottles could be measured through GasBench II-IRMS. No modifications were made to this procedure.

3.5.2. The calculations of DOC contents and $\delta^{13}\text{C}_{\text{DOC}}$ values in natural water samples

After the pre-treatment following the above procedure, the DOC contents and $\delta^{13}\text{C}_{\text{DOC}}$ values of the raw water samples could be obtained using reference solutions with known DOC contents and $\delta^{13}\text{C}_{\text{DOC}}$ values. It was worth noting that the water samples and reference solutions should be dealt with under the same volume of 40 mL. DOC blank examples with the distilled water were also prepared to get the CO_2 peak background values. After that, the CO_2 peak from reference solutions represented the mixing of DOC from reference materials and distilled water. Consequently, the linear relationship between DOC contents and CO_2 peak from only reference materials was established after excluding the CO_2 peak from distilled water. This linear relationship could be utilized to calculate the DOC content in natural water samples due to no distilled water in them. For $\delta^{13}\text{C}_{\text{DOC}}$ values of water samples, a linear relationship between $\delta^{13}\text{C}_{\text{DOC}}$ raw values and true values was established by two reference materials, including sucrose and potassium hydrogen phthalate with $\delta^{13}\text{C}$ values of $-12.9\text{‰} \pm 0.2\text{‰}$ and $-29.1\text{‰} \pm 0.2\text{‰}$, respectively (Table 1). Similar to DOC content determination, the $\delta^{13}\text{C}_{\text{DOC}}$ values of reference solutions covered the $\delta^{13}\text{C}$ values of both solid references and distilled water, so the above linear relationship was obtained only when $\delta^{13}\text{C}$ values of distilled water were excluded.

3.6. Effect of improved method on DOC content and $\delta^{13}\text{C}_{\text{DOC}}$ value of natural water samples

The DOC concentration and isotope values of standard and some natural samples were determined and analyzed by the improved and the original method, respectively. The original data and correction results of isotope ratios of Gas Bench II and EA of KHP and SUC are plotted in Figure 6. In the improved method and the original method, the lower the $\delta^{13}\text{C}$ value is affected by the blank logarithmic form. The corrected results are closer to EA values, and the blank effects on small samples are drastically eliminated. In the improved method, the standard deviation of the corrected isotope ratio is less than 0.2‰. The blank effect and system error were corrected by this method. The dilution curve of the standard sample showed that the method had high precision (0.2‰) and a low detection limit (8 μgC). The concentration of natural water was 0.54–7.88 $\text{mg}\cdot\text{L}^{-1}$, and the concentration test accuracy was not more than 0.12 $\text{mg}\cdot\text{L}^{-1}$ (Supplementary material, Table S5). The accuracy of $\delta^{13}\text{C}$ of natural water samples obtained by the improved method was not more than 0.2‰, which was better than the accuracy reported previously (0.2–0.46‰) (Lang *et al.* 2012; Zhou *et al.* 2015). In addition, the DOC content and $\delta^{13}\text{C}$ values had good repeatability.

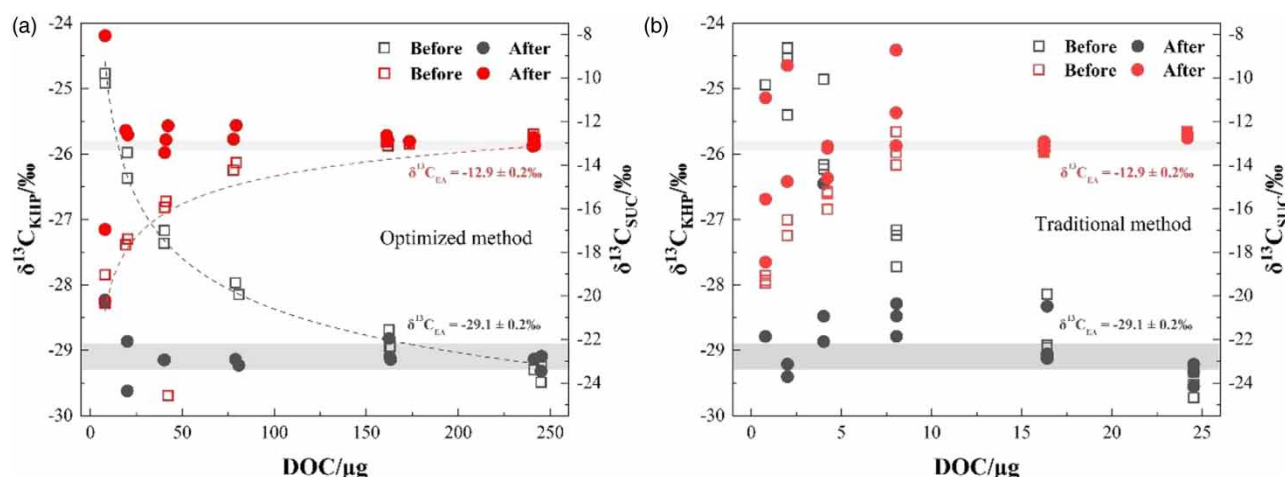


Figure 6 | The $\delta^{13}\text{C}_{\text{GB}}$ values of DOC standard samples (KHP and SUC) obtained by the improved method (a) and the traditional method (b) varied with carbon content. In this analysis, the carbon concentration of the reference solution was the same (0.2–6 $\text{mg}\cdot\text{L}^{-1}$). The shadow parts in the figure represent its $\delta^{13}\text{C}_{\text{EA}}$ values. The lower the $\delta^{13}\text{C}$ values, the greater the impact of the blank.

3.6.1. Limitations and considerations

The effective application of stable isotope analysis technique relies on achieving high precision and accuracy in determining isotopic composition (Lang *et al.* 2012; Zhou *et al.* 2015). However, there are certain limitations in the pre-treatment methods for stable carbon isotopes. For instance, this study identified that nitrogen conversion in samples may lead to carbon isotope fractionation, thereby causing varying degrees of impact on the results. This limitation has not been sufficiently addressed and necessitates further investigation for resolution. Furthermore, potential errors stemming from sample preparation and handling procedures can pose challenges in interpreting the complexities of environmental factors and biological processes. Therefore, prior to conducting this research methodology, several crucial considerations need to be taken into account. First, it is imperative to exercise caution during the constant heating process at 100 °C to prevent loosening or fracturing of bottle caps, as this could lead to the ingress of atmospheric CO₂ into the bottles or the escape of bottle gases, potentially influencing the outcomes. Second, fluctuations in DOC levels may introduce deviations in DOC quantification within the test solution, with an increase in the test solution volume exacerbating the impact of background effects. Hence, ensuring a sufficiently low DOC content in the blanks and deducting the corresponding background values during data analysis are pivotal measures.

4. CONCLUSIONS

Due to the low accuracy of the determination of DOC contents and $\delta^{13}\text{C}$ values of natural water with low DOC concentration, we increased the volume of water samples to be oxidized by the WCO method. Nitrogen gas purging in an ice bath within 2 h was used to remove DIC, and its effectiveness was guaranteed by DIC solutions and DIC/DOC mixed solutions with known $\delta^{13}\text{C}$ values. The distilled water had low DOC contents and could dissolve solid standard references. After excluding the background values of DOC contents and $\delta^{13}\text{C}$ values from distilled water, good linear relationships between DOC amount and CO₂ produced by WCO, and $\delta^{13}\text{C}$ raw values and true values were established to determine the DOC contents and $\delta^{13}\text{C}$ values of natural water samples under the same conditions of 40 mL solutions. Our improved pre-treatment methods obtained good accuracy and stability on natural water samples with low DOC concentrations of $\sim 0.5 \text{ mg L}^{-1}$. Moreover, this method saved much pre-treatment time and realized batch processing of natural water samples through three-way valves in the purge process.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

REFERENCES

- Assayag, N., Rive, K., Ader, M., Jezequel, D. & Agrinier, P. 2006 Improved method for isotopic and quantitative analysis of dissolved inorganic carbon in natural water samples. *Rapid Communications in Mass Spectrometry* **20**, 2243–2251. <https://doi.org/10.1002/rcm.2585>.
- Bao, H., Wu, Y., Zhang, J., Deng, B. & He, Q. 2014 Composition and flux of suspended organic matter in the middle and lower reaches of the Changjiang (Yangtze River)-impact of the Three Gorges Dam and the role of tributaries and channel erosion. *Hydrological Processes* **28**, 1137–1147. <https://doi.org/10.1002/hyp.9651>.
- Barnes, R. T. & Raymond, P. A. 2009 The contribution of agricultural and urban activities to inorganic carbon fluxes within temperate watersheds. *Chemical Geology* **266**, 318–327. <https://doi.org/10.1016/j.chemgeo.2009.06.018>.

- Das, A., Krishnaswami, S. & Bhattacharya, S. K. 2005 Carbon isotope ratio of dissolved inorganic carbon (DIC) in rivers draining the Deccan Traps, India: Sources of DIC and their magnitudes. *Earth and Planetary Science Letters* **236**, 419–429. <https://doi.org/10.1016/j.epsl.2005.05.009>.
- David, M. B., Vance, G. F. & Kahl, J. 1992 Chemistry of dissolved organic carbon and organic acids in two streams draining forested watersheds. *Water Resources Research* **28**, 389–396. <https://doi.org/10.1029/91WR02180>.
- Du, Y. & Song, H. 2020 Refined protocol for the $\delta^{13}\text{C}$ analysis of calcite and dolomite in carbonate mixture samples. *Rapid Communications in Mass Spectrometry* **34**. <https://doi.org/10.1002/rcm.8743>.
- Federherr, E., Cerli, C., Kirkels, F. M., Kalbitz, K., Kupka, H. J., Dunsbach, R., Lange, L. & Schmidt, T. C. 2014 A novel high-temperature combustion based system for stable isotope analysis of dissolved organic carbon in aqueous samples. I: Development and validation. *Rapid Communications in Mass Spectrometry* **28** (23), 2559–2573. <https://doi.org/10.1002/rcm.7052>.
- Gandhi, H., Wiegner, T. N., Ostrom, P. H., Kaplan, L. A. & Ostrom, N. E. 2004 Isotopic (^{13}C) analysis of dissolved organic carbon in stream water using an elemental analyzer coupled to a stable isotope ratio mass spectrometer. *Rapid Communications in Mass Spectrometry* **18**, 903–906. <https://doi.org/10.1002/rcm.1426>.
- Ge, T., Xue, Y., Jiang, X., Zou, L. & Wang, X. 2020 Sources and radiocarbon ages of organic carbon in different grain size fractions of Yellow River-transported particles and coastal sediments. *Chemical Geology* **534**. <https://doi.org/10.1016/j.chemgeo.2019.119452>.
- Kang, S., Kim, J.-H., Kim, D., Song, H. & Shin, K. H. 2019 Temporal variation in riverine organic carbon concentrations and fluxes in two contrasting estuary systems: Geum and Seomjin, South Korea. *Environment International* **133**, 105126. <https://doi.org/10.1016/j.envint.2019.105126>.
- Keys, K. A. 1934 Methods for the determination of dissolved organic carbon and nitrogen in sea water. *Biological Bulletin* **67**, 132–144. <https://doi.org/10.2307/1537488>.
- Kirschbaum, M. U. F., Zeng, G., Ximenes, F., Giltrap, D. L. & Zeldis, J. R. 2019 Towards a more complete quantification of the global carbon cycle. *Biogeosciences* **16**, 831–846. <https://doi.org/10.5194/bg-2018-436>.
- Krishnamurthy, R. V. & Atekwana, E. A. 1997 A simple, inexpensive carbonate-phosphoric acid reaction method for the analysis of carbon. *Analytical Chemistry* **69**, 4256–4258. <https://doi.org/10.1021/ac9702047>.
- Lang, S. Q., Lilley, M. D. & Hedges, J. I. 2007 A method to measure the isotopic (^{13}C) composition of dissolved organic carbon using a high temperature combustion instrument. *Marine Chemistry* **103**, 318–326. <https://doi.org/10.1016/j.marchem.2006.10.002>.
- Lang, S. Q., Bernasconi, S. M. & Früh-Green, G. L. 2012 Stable isotope analysis of organic carbon in small (μgC) samples and dissolved organic matter using a GasBench preparation device. *Rapid Communications in Mass Spectrometry* **26**, 9–16. <https://doi.org/10.1002/rcm.5287>.
- Li, Y., Wang, M., Zhang, D., Wang, F. & Jiang, H. 2024 The impacts of water-sediment regulation on organic carbon in the Yellow River. *Science of The Total Environment* **918**. <https://doi.org/10.1016/j.scitotenv.2024.170721>.
- Mckenna, J. H. & Doering, P. H. 1995 Measurement of dissolved organic carbon by wet chemical oxidation with persulfate: Influence of chloride concentration and reagent volume. *Marine Chemistry* **48**, 109–114. [https://doi.org/10.1016/0304-4203\(94\)00049-j](https://doi.org/10.1016/0304-4203(94)00049-j).
- Menzel, D. W. & Vaccaro, R. F. 1964 The measurement of dissolved organic and particulate carbon in seawater. *Limnology and Oceanography* **9**, 138–142. <https://doi.org/10.4319/lo.1964.9.1.0138>.
- Osburn, C. L. & St-Jean, G. 2007 The use of wet chemical oxidation with high-amplification isotope ratio mass spectrometry (WCO-IRMS) to measure stable isotope values of dissolved organic carbon in seawater. *Limnology and Oceanography: Methods* **5**, 296–308. <https://doi.org/10.4319/lom.2007.5.296>.
- Rock, L. & Mayer, B. 2006 Tracing nitrates and sulphates in river basins using isotope techniques. *Water Science and Technology* **53**, 209–217. <https://doi.org/10.2166/wst.2006.314>.
- Romankevich, E. A. 1984 *Geochemistry of Organic Matter in the Ocean*. Springer-Verlag Berlin–Heidelberg, Germany.
- Sharp, J. H. 1973 Total organic carbon in seawater-comparison of measurements using persulfate oxidation and high temperature combustion. *Marine Chemistry* **1**, 211–229. [https://doi.org/10.1016/0304-4203\(73\)90005-4](https://doi.org/10.1016/0304-4203(73)90005-4).
- Skopintsev, B. 1976 On the determination of organic carbon in chloride rich water by the persulfate method. *Oceanology* **16**, 630–633.
- Skopintsev, B., Timofeyeva, S. & Verzhinina, O. 1966 Organic carbon in the equatorial and southern Atlantic and in the Mediterranean. *Oceanology* **6**, 201–210. <https://doi.org/10.1016/j.gloplacha.2003.08.002>.
- Skrzypek, G. 2013 Normalization procedures and reference material selection in stable HCNOS isotope analyses: An overview. *Analytical and Bioanalytical Chemistry* **405**, 2815–2823. <https://doi.org/10.1007/s00216-012-6517-2>.
- Skrzypek, G. & Ford, D. 2019 Reference materials selection for the stable carbon isotope analysis of dissolved carbon using a wet oxidation system. *Rapid Communications in Mass Spectrometry* **33**, 473–481. <https://doi.org/10.1002/rcm.8351>.
- Skrzypek, G., Sadler, R. & Paul, D. 2010 Error propagation in normalization of stable isotope data: A Monte Carlo analysis. *Rapid Communications in Mass Spectrometry* **24**, 2697–2705. <https://doi.org/10.1002/rcm.4684>.
- Spötl, C. 2005 A robust and fast method of sampling and analysis of $\delta^{13}\text{C}$ of dissolved inorganic carbon in ground waters. *Isotopes in Environmental and Health Studies* **41**, 217–221. <https://doi.org/10.1080/10256010500230023>.
- Wen, Z., Song, K., Liu, G., Lyu, L. & Du, J. 2020 Characterizing DOC sources in China's Haihe River basin using spectroscopy and stable carbon isotopes. *Environmental Pollution* **258**, 113684. <https://doi.org/10.1016/j.envpol.2019.113684>.
- Wilson, R. F. 1961 Measurement of organic carbon in seawater. *Limnology and Oceanography* **6**, 259–261. <https://doi.org/10.1021/ba-1975-0147.ch013>.

- Xu, H., Xu, X., Chen, L., Guo, J. & Wang, J. 2022 A novel cryogenic condensation system combined with gas turbine with low carbon emission for volatile compounds recovery. *Energy* **248**. <https://doi.org/10.1016/j.energy.2022.123604>.
- Yi, Y., Zhong, J., Bao, H., Mostofa, K. M. G., Xu, S., Xiao, H. Y. & Li, S. L. 2021 The impacts of reservoirs on the sources and transport of riverine organic carbon in the karst area: A multi-tracer study. *Water Research* **194**, 116933. <https://doi.org/10.1016/j.watres.2021.116933>.
- Yu, K., Gan, Y., Zhou, A., Han, L. & Liu, Y. 2015 A persulfate oxidation method for stable isotope analysis of dissolved organic carbon and the influence of inorganic ions on the results. *International Journal of Mass Spectrometry* **392**, 63–68. <https://doi.org/10.1016/j.ijms.2015.09.006>.
- Zhang, J., Quay, P. D. & Wilbur, D. O. 1995 Carbon-isotope fractionation during gas-water exchange and dissolution of CO₂. *Geochimica Et Cosmochimica Acta* **59**, 107–114. [https://doi.org/10.1016/0016-7037\(95\)91550-D](https://doi.org/10.1016/0016-7037(95)91550-D).
- Zhang, W., Zhang, Y.-L., Cao, F., Xiang, Y., Zhang, Y., Bao, M., Liu, X. & Lin, Y.-C. 2019 High time-resolved measurement of stable carbon isotope composition in water-soluble organic aerosols: Method optimization and a case study during winter haze in eastern China. *Atmospheric Chemistry and Physics* **19**, 11071–11087. <https://doi.org/10.5194/acp-19-11071-2019>.
- Zhang, D., Zhao, Z.-Q., Peng, Y., Fan, B., Zhang, L., Li, J. & Chen, A. 2020a Sulfur cycling in the Yellow River and the sulfate flux to the ocean. *Chemical Geology* **534**. <https://doi.org/10.1016/j.chemgeo.2019.119451>.
- Zhang, L., Fang, W., Li, X., Lu, W. & Li, J. 2020b Strong linkages between dissolved organic matter and the aquatic bacterial community in an urban river. *Water Research* **184**, 116089. <https://doi.org/10.1016/j.watres.2020.116089>.
- Zhou, Y., Guo, H., Lu, H., Mao, R., Zheng, H. & Wang, J. 2015 Analytical methods and application of stable isotopes in dissolved organic carbon and inorganic carbon in groundwater. *Rapid Communications in Mass Spectrometry* **29**, 1827–1835. <https://doi.org/10.1002/rcm.7280>.

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