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Could wastewater analysis be a useful tool for China?

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Applying a new method for direct collection, volume quantification and determination of N_2 emission from water

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ABSTRACT

The emission of N_2 is important to remove excess N from lakes, ponds, and wetlands. To investigate the gas emission from water, Gao et al. (2013) developed a new method using a bubble trap device to collect gas samples from waters. However, the determination accuracy of sampling volume and gas component concentration was still debatable. In this study, the method was optimized for *in situ* sampling, accurate volume measurement and direct injection to a gas chromatograph for the analysis of N_2 and other gases. By the optimized new method, the recovery rate for N_2 was 100.28% on average; the mean coefficient of determination (R^2) was 0.9997; the limit of detection was 0.02%. We further assessed the effects of the new method, bottle full of water, *vs.* vacuum bag and vacuum vial methods, on variations of N_2 concentration as influenced by sample storage times of 1, 2, 3, 5, and 7 days at constant temperature of 15°C, using indices of averaged relative peak area (%) in comparison with the averaged relative peak area of each method at 0 day. The indices of the bottle full of water method were the lowest (99.5%–108.5%) compared to the indices of vacuum bag and vacuum vial methods (119%–217%). Meanwhile, the gas chromatograph determination of other gas components (O_2, CH_4, and N_2O) was also accurate. The new method was an alternative way to investigate N_2 released from various kinds of aquatic ecosystems.

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Introduction

The nitrogen cycle and dynamics are both essential ecosystem functions for healthy aquatic environments (Leon et al., 2003). Dinitrogen (N_2) and nitrous oxide released from the processes of denitrification are key aspects in the nitrogen cycle and dynamics (James et al., 2011), especially as related to the management of eutrophic water bodies (Zhong et al., 2010). With accelerated eutrophication all over the world, N_2 and N_2O ebullitions from denitrification processes in waters have attracted great interest (Gao et al., 2012; Hamersley and Howes, 2005; Hwang and Hanaki, 2000) and are important topics for water resources and environmental management as well as for the evolution of aquatic ecosystems (James et al., 2011; Lu et al., 2009; Magalhães et al., 2005).

In studies involving gas samples, vacuum airbags and vacuum vials are usually used for sample collection; and syringe and needle samplers are used as intermediate transport tools (Cai et al., 2013; Sun et al., 2002; Smith and Lewis, 1992; Zhu et al., 2005). However, vacuum airbags and vials are not easily used for the quantitative measurement of ebullition by weight or by volume. Furthermore, needle samplers are frequently blocked by impurities and algae

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within the sampling processes (Chanton et al., 1989; Huttunen et al., 2001; Zhu et al., 2005) since many investigations of ebullition from waters have been conducted at the sediment–water interface (Higgins et al., 2008; Leon et al., 2003). The syringe method for determining gas volume and transferring gas samples has been used in research on methane, nitrous dioxide and other gases (Sun et al., 2002; Smith et al., 2000), while additional gas transport processes may increase the risk of contamination because N_2 gas ebullition from waters may be easily contaminated due to the high concentration of nitrogen gas in the air (~78% N_2 and 21% O_2) with high diffusive capability and permeability. Recently, Gao et al. (2013) employed an improved bubble trap device to investigate the N_2 and N_2O ebullition from eutrophic water. All the gas, including ebullition and diffusive gas released from the water column and sediment (Mccutchan et al., 2003), was collected by the trap devices and eventually imported into the sampling bottle (Gao et al., 2013). However, because the improved bubble trap device method altered the natural process of gas release from the water–air interface and gas samples were stored in water bottles, the determination accuracy of the sample volume and component concentration was debatable.

The gas chromatograph (GC) is a common analytical tool for component analysis of gas samples. To inject samples into a GC, the headspace method is mature and was primarily developed for analyzing volatile gases in liquids (AEPA, 1989; Bicchi et al., 2008, 2012; Christensen and Reineccius, 1992; Ho et al., 2012; Snow and Slack, 2002; Stalder et al., 1995; Yang et al., 2002), which may not be suitable for analyzing the gas samples collected by Gao's method. Traditional manual injection with a syringe can achieve fairly accurate N_2 measurement (Stalder et al., 1995), but it is complex, risky and unnecessary to transfer the gas samples from sampling bottles into syringes for injection of samples to the GC. A simple, convenient and precise method for the analysis of N_2 of large quantities of gas samples was still needed. A modern GC is equipped with valves and loops to provide accurate injection sample volume, accompanied with precise detectors for analysis of gas components such as N_2, oxygen, nitrous oxide and methane (Klifta et al., 2008; Ma et al., 1996). The method of online injection assembles gas sample generator devices with a GC, which has limited application areas and has been abandoned (Carmichael and Holmes, 1990). Therefore, developing a new and applicable transfer method is important for accurate determination of N_2 and other gases in sampling bottles.

In this study, we further verified gas sample volume measurement, developed and optimized gas transfer, purging and injecting methods and evaluated the integrity of Gao's method and GC analysis. The research conclusions were expected to confirm the feasibility, reliability and stability of the new bottle full of water (BFW) method before high volume use.

1. Materials and methods

1.1. Bottle full of water device, sampling and gas volume measurement methods

The bottle used was a narrow-necked glass bottle (Fig. 1) sealed with a PTFE/silicone stopper. There was one intake tube (Φ 4 mm, glass) and one outlet tube (Φ 2 mm, glass) pierced through the stopper. The intake tube extended to the bottom of the bottle and 100 mm outside of the stopper; the outlet tube extended 8 mm into the bottle, measured from the inner side of the stopper, and 20 mm outside of the stopper. The outside parts of the tubes were connected to a thick-wall silicone tube (2.4 mm) with high quality gas-tight fastenings (Jacinthe and Dick, 1996). Before collecting gas samples from the water body, the bottle and the silicone tubes were filled with pure water in the lab; and the open ends of the silicone tubes were sealed by clamps and weighed (accurate to ±0.01 g). At the time of sampling, the bottle was inverted and held above the gas source; the intake tube was connected to the gas source with a high quality gas-tight fastening; the clamped open end of the outlet tube was placed in the water and the clamps at the open ends of the intake and outlet tubes were released. In this procedure, gas was driven by gravity into the bottle, meanwhile an equivalent volume of water flowed away. After sampling, the open ends of the two silicone tubes were clamped again. Then, the bottle remained in the inverted position and was transported to the lab. The gas samples were analyzed immediately.

The mass of the bottle including the collected gas sample and the tubes was weighed; and the volume of the water loss was estimated. The volume of gas sample in the bottle was calculated as follows:

$$V = \frac{W_1 - W_2}{D} \quad (1)$$

$$D = 1 - \frac{t + 288.94}{508929.20(t + 68.13)}(t - 3.99)^2 \quad (2)$$

where, V (mL) is the gas volume at 15°C; W_1 (g) is the weight of the pre-filled bottle before gas collection; W_2 (g) is the weight of the bottle after gas collection; and D is the water density at 15°C; The water density (D) was calculated as a function of temperature (0°C ≤ t ≤ 50°C) (McCutcheon et al., 1993); t is the water temperature in a sample bottle.

1.2. Method for direct injection of gas sample into GC

The injection system was set up according to Fig. 1. Two 500-mm-height, 25-L water jars were prepared and filled with pure water, which kept the inner jar water at a constant level by water supply and drainage *via* a pump in the outer jar and water channel between the two jars. The inner jar was equipped with a valve for outflow control on the side wall near the bottom. The valve was connected to the intake tube of the sampling bottle in an upright position without trapping any air bubbles inside the tube. The valve was opened to let water flow freely from the jar into the bottom of the sample bottle to increase the pressure inside the bottle. The GC sampling subsystem was connected *via* gas-tight connections to the outlet tube of the sampling bottle through a thick-wall silicone tube including a dry silica gel tube and a peristaltic pump. In operation, the peristaltic pump was started first; then the clamp at the outlet tube side of the sampling bottle was released; the leading gas in the sampling system was purged before the injection of sample gas. The total volume of the outlet tube and dry silica gel tube between the sampling bottle and the GC was 10 mL.

1.3. GC chemical analysis

A gas chromatograph (GC-2010, Shimadzu, Kyoto, Japan), equipped with a thermal-conductivity detector (TCD) and two molecular sieve columns plus a Ni-63 electron capture detector (ECD, Shimadzu, Kyoto, Japan) with a ten-way valve and a 1-mL sample loop, was used for all analysis. The sample injection system was connected to the sampling bottle (see details in Section 1.2) *via* thick-wall silicone tube. The

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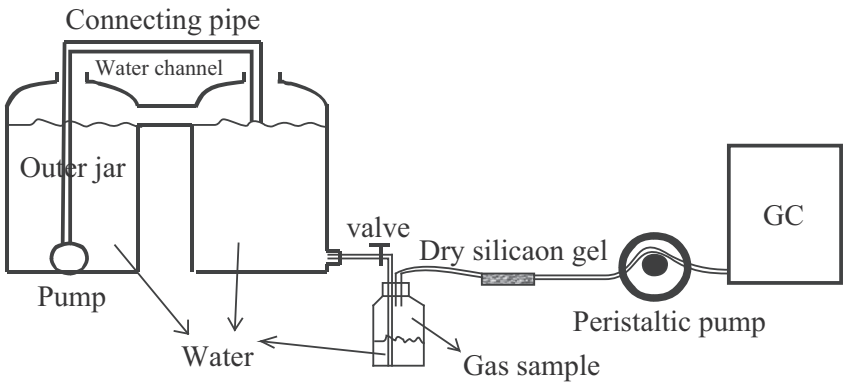

Fig. 1 – Sketch of device and connections for gas purging and injection into GC (gas chromatograph) at analysis.

injection process consisted of two steps: first, the whole injection system including sample loop was purged by gas from the sampling bottle through the injection port; second, the gas sample in the sample loop with constant volume (1 mL) was transferred into the GC.

N_2, CO_2, CH_4, and O_2 were detected using the thermal-conductivity detector (TCD) with highly purified hydrogen as the carrier gas (8 mL/min). Columns used involved a molecular sieve column, Φ2 mm × 3 m Porapak Q column and Φ2 mm × 4 m Porapak QS column (Shimadzu, Shanghai, China) with temperature at 70°C. The temperature of the detector was set at 200°C with an electrical current of 70 mA.

N_2O was detected using an ECD detector; two separation columns, specifically Φ2 mm × 2 m Porapak Q and Φ2 mm × 6 m Porapak QS, were used. The temperature of the column was set at 70°C; and the temperature of the detector was set at 300°C with an electrical current of 1.0 nA. The makeup gas was composed by 5% CH_4/Ar with flow rate of 30.0 mL/min. The contents of gases were determined by peak area normalization.

1.4. Evaluation of analytical precision

1.4.1. Gas volume in sample bottle and application in practice

There were two sets of experiments conducted for: (1) checking accuracy of the measurement of water volume being replaced by the sample gas; (2) actually collecting gas ebullition from eutrophic water and measuring the volume collected.

In the first set of the experiments, bottles were filled with pure water and weighed first; and then, 0, 50, 100, 150, 200, 250, and 300 mL of air were injected into each (treatment) of the bottle with three replicates. The weight losses of the bottles were determined by electronic balance; and the volumes of water being replaced by the injected air were calculated using Eqs. (1) and (2). The temperature was maintained at 15°C.

In the second set of experiments, four batches of gas samples were collected with three replicates for each batch. The duration of gas collecting time for each batch was maintained 24-hr in a local pond in Nanjing, China on 20–21 June and 7–8, 8–9, and 22–23 September in 2012. The pond was approximately 5400 m^2 in area and 1.5 to 2 m in depth.

1.4.2. Purging durations with variation of peristaltic pump speed

The total purging volume of helium (He 99.99%) during the clean-up process was determined using two sets of experiments: (E1) a fixed purging duration of 100 sec with variable peristaltic pump speeds of 10-, 20-, 50-, 80-, and 100 r/min; (E2) a fixed pump speed of 50 r/min with variable purging durations of 10, 30, 60, 100, and 120 sec. All treatments with four replicates were prepared according to the method mentioned in Section 1.4.1 but with the gas being He (99.99%). The time of releasing the clamp at the opening end of the gas tube was considered to be the starting time (Fig. 1). When the desired purging duration had been met, the clamp was closed; the pump was stopped; and the outflow control valve was closed. The purging volumes were calculated according to Section 1.1.

1.4.3. Optimization of peristaltic pump speed and running time

The operation parameters were optimized by detecting peak areas of He (99.99%) samples from the sampling bottles. First, the recovery rates of He at different purging volumes of variable peristaltic pump speed based on the results of E1 in Section 1.4.2 were calculated. Next, the recovery rate of He at different purging volumes at 50 r/min with different peristaltic pump running times based on the results of E2 in Section 1.4.2 were calculated. The criterion of the optimization was set on the maximum recovery rate of He (99.99%) gas at the shortest purging time.

1.4.4. Gas concentration linearity and recovery

Six standard gases including air, He (99.99%), standard mixed gas No. 1 (N_2 15.5%, CO_2 2.1%, CH_4 48.0%), standard mix No. 2 (N_2 91.2%, O_2 8.8%), standard mix No. 3 (N_2 24.8%, CO_2 28.9%, CH_4 46.3%) and nitrous oxide (N_2O, 1 μL/L) were prepared for the experiment. All the standard gases were held in cylinders and purchased from the 55th institute of China Light and Power Group Corporation (Nanjing, China). To evaluate optimized operation settings for different gases, sampling bottles were filled with pure water and weighed, and then the standard gases in cylinders were filled into the sampling bottles through the inlet tube, meanwhile waters in the bottles flowed out freely. Altogether, twenty-four bottles, accounting for four replicates of each gas type, were prepared. The samples were analyzed immediately after preparation. To assess possible errors introduced by the above procedures, direct injections (without silicone tube and peristaltic pump) of air, He (99.99%) and standard mixed gases were carried out as the standard treatment method.

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1.4.5. *Comparison of different sampling devices*

Six sets, one set for each designed storage time of vacuum bag (VB), vacuum vial (VV) and BFW devices, were prepared with N_2 (99.99%) gas samples with three replicates. The devices were stored at 15°C for 0, 1, 2, 3, 5, and 7 days. At the end of each designed storage time, one set of the devices with their N_2 contents was analyzed according to Section 1.3. The indices of the average relative peak areas (ARPA, %) at the different storage times (1, 2, 3, 5, 7 days) compared to the ARPA at day 0 were evaluated among the different sampling devices using the lowest deviation as the criterion for good stability of the devices.

1.5. *Statistical analysis*

SigmaPlot 12.0 (Systat Software, Inc., Chicago, IL, USA) was used for statistical analysis and data plotting. The peristaltic pump speed and running duration data were fitted using a linear regression. The optimized pump speed and running duration were selected based on multivariate linear regression. Differences between treatments were compared using a paired *t*-test with significance at $p < 0.05$ (2-tailed).

2. Results and discussion

2.1. *Gas sample volume measurement and application in practice*

Because gas collection was based on the water–gas displacement principle, *i.e.*, a gas sample volume was theoretically equal to the water volume being displaced by the gas sample, meanwhile the water volume was calculated based on the water density at a given temperature (Eqs. (1) and (2)) (t = 15°C in this study), measurements were made to confirm the accuracy of this approach. The result showed a strong correlation between the gas volume injected into the bottle and the volume of the water being replaced ($R^2 = 1.0000$, $p < 0.01$, Table 1); and there was no significant difference between gas volume and water volume ($p > 0.05$). This finding suggested that the volume measurement method was accurate and reliable. The volumes of gas samples collected at the different temperatures could be calculated using Eqs. (1) and (2) by the water volume being displaced with the water density at the corresponding temperatures.

Ebullition from waters may vary widely according to limnological and hydrological properties or environmental characteristics. The standard deviation (SD) of the observed data in the field could range from 50% to 81% for sampling in rivers (Smith et al., 2000), or from 28.33% to 31.53% for sampling in lakes (Smith and Lewis, 1992). Measurements from a large lake even produced standard deviations ranging from 22.62% to 131.80% (Keller and Stallard, 1994). After careful exclusion of natural factors by limiting the survey area among replicates (<10 × 10 m) and by avoiding extreme weather conditions, such as high winds or storms, the SD among replicates observed in our investigation varied from 2.94% to 10.23% in the overall handling and measurement processes. The BFW device was employed in gas sampling at various water temperatures in the local pond; and the SD of the observed volumes was less than 7.80% (Table 2). None of the sample volumes exceeded the limitation (500 mL) of the selected bottle type. Although large standard deviations may result from the variation of biological processes in the sediment, our results indicated that the data produced by the BFW device was acceptable in terms of the variation among replicates.

2.2. *Purging volume of GC injection system as a function of peristaltic pump speed and duration*

The purging volume, which was calculated using Eqs. (1) and (2), is important to ensure a complete purge of air in the injection tube of a GC. In this study, the purging gas before the injection of gas samples from the sampling bottles to the GC was driven dynamically by a peristaltic pump and water pressure from a water jar (Fig. 1). The water pressure from the water jar was sufficient and stable so that the peristaltic pump speed and running duration were critical to the purging volume. When running duration was set at 100 sec, the purging volumes ranged from 13.66 ± 0.24 to 107.43 ± 0.81 mL ($R^2 = 0.9975$, $p < 0.001$) for pump speeds of 10 to 100 r/min. When the peristaltic pump speed was set at 50 r/min, the purging volumes ranged from 11.21 ± 0.44 to 67.61 ± 0.58 mL ($R^2 = 0.9976$, $p < 0.001$) for purging durations of 10 to 120 sec (Fig. 2). The peristaltic pump speed and running duration were strongly and positively correlated with the purging volume, suggesting that the sample-purging-injection device of the BFW and the method were stable.

Table 1 – Gas volume injected into bottle, weight of water displaced by injected air, and volume of displaced water.

Gas volume injected into bottle (mL) [a]	Weight of the replaced water (g) [b]	Volume of the replaced water (mL) [c]
0.00	0	0
100.00	99.90 ± 0.52	99.99 ± 0.52
200.00	199.78 ± 0.84	199.96 ± 0.84
300.00	299.71 ± 0.95	299.98 ± 0.95
400.00	399.68 ± 0.58	400.04 ± 0.58
500.00	499.59 ± 0.67	500.04 ± 0.67

[a] Injected by precision syringe.
[b] Measured by electrical balance (mean ± SD). SD = standard deviation
[c] Calculated using Eqs. (1) and (2) temperature = 15°C (mean ± SD).

Table 2 – Gas sample measurement (volume) for different average daily water temperatures. [a]

Gas collecting location (Water depth 1.40 m)	Average daily water temperature (°C)	Total volume (mL/24-hr)	RSD (Total volume)
50 mm above sediment	32.00	353.90 ± 15.11	4.27%
At water surface	27.50	407.32 ± 6.53	1.60%
At water surface	24.00	209.91 ± 16.45	7.84%
At water surface	25.50	314.07 ± 22.70	7.23%

[a] Nutrient concentrations of water (mean ± SD, mL/L): nitrate 1.71 ± 0.24, nitrite 0.28 ± 0.04, ammonia 0.19 ± 0.09, total nitrogen 3.33 ± 0.20, total phosphorous 0.11 ± 0.02; $n = 3$ for all data for reference. RSD: relative standard deviation, $n = 4$.

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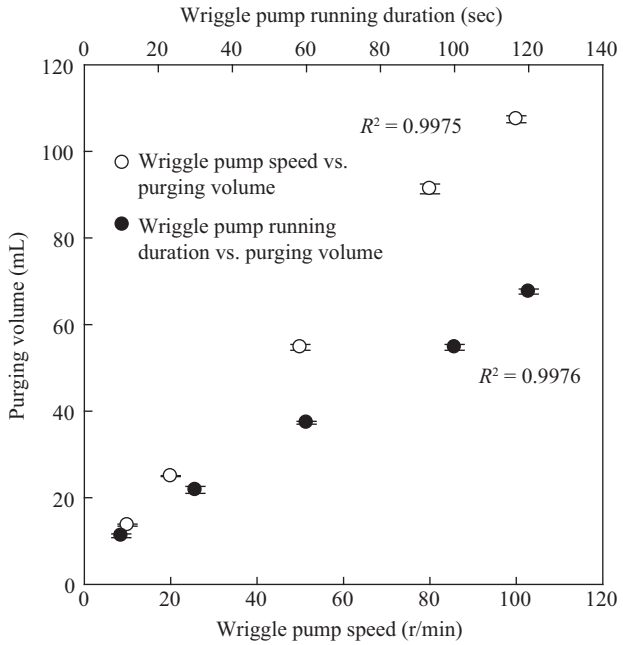

Fig. 2 – Purging volume as a function of peristaltic pump speed and running duration.

2.3. Optimized purging volume using peristaltic pump running speed and duration

To optimize the purging parameters, He gas was chosen as the standard gas because of its lower solubility in water. A stable peristaltic pump speed and the shortest duration while still providing a complete purge of the leading air in the injecting subsystem were the primary criteria for selecting parameters for GC analysis. At 50 r/min, the purging volumes were 11.21, 21.80, 37.33, 54.73, and 67.61 mL for pump running durations of 10, 30, 60, 100, and 120 sec, respectively (Fig. 2). The peak areas of He increased with increasing purging volumes (Fig. 3, $p < 0.001$); and the highest peak area was achieved at a purging volume of 67.61 mL, and there was no significant difference between the peak areas at purging volumes of 54.73 and 67.61 mL. The lowest purging volume allowing the complete purge of air in the injection tube was 60 ± 6 mL.

If a 60-mL purging volume was introduced into the GC, then five parameter combinations (10-550; 20-300; 50-110; 80-66; 100 r/min-55 sec) would be satisfactory according to the results from Section 2.2. The results for the He peak areas detected under the five parameter combinations mentioned above revealed that the He peak areas detected using 100 r/min-55 second combination was 98.68% of the standard value (Fig. 3). There was no significant difference between the He peak areas detected using 80 r/min-66 sec and 100 r/min-55 sec. Thus, the optimized parameters of speed at 100 r/min and duration of purging for 55 sec were selected.

2.4. Gas recovery precision and application to real samples

Using the optimized parameters, the N_2 in the air and in the calibrating gas mixtures was sampled and analyzed by the BFW device method for comparison with the N_2 contents of the standard method. The contents of the standard gas mixtures were selected based on an averaged gas composition obtained from a previous local study (Gao et al., 2012, 2013). The coefficient of determination (R^2) of N_2 was 0.9997 (Table 3). The limit of detection (LOD) was evaluated by the signal-to-noise (S/N) of the measurements using the lowest-content detected peak. The N_2 S/N was 0.02%. The recovery was determined as the ratio of the detected peak area of the BFW device to that of the standard method. The recoveries of the detected N_2 peak areas were 99.80% for N_2 from air and 100.28% for N_2 from standard gas mixtures. The thick-wall silicone tube has low permeability (Jacinthe and Dick, 1996) and the purging time was short (under 1 min), so the diffusion between the sample gas inside the tubes and air outside the tubes could be considered minimal and neglectable. These results suggested that the BFW device method was accurate and reliable for the determination of N_2 in ebullitions from eutrophic waters.

For other gases (He, O_2, CH_4, CO_2 and NOx), the recovery rates generally ranged from 98.50% to 100.28%, except for carbon dioxide (89.50%) (Table 3). All RSD (relative standard deviation) values were very small (<0.94%). The LODs were 0.02%–0.59%, except for oxygen (1.02%) and nitrous oxide (0.01 μL/L). These results suggested that the BFW device method was also reliable for the determination of ebullition from waters, except for CO_2. The linearity of the other gases (He, O_2, CH_4, CO_2 and NOx) was not further investigated because the linearity range of the TCD detector was wider (Cowper, 2000) and these gases were beyond the scope of this study.

To investigate the feasibility of the new method, real gas samples from 50 mm above sediment were collected and analyzed. The concentrations of the gas samples were 2.07% O_2, 18.35% N_2, 1.26% CO_2 and 78.75% CH_4. The results showed that all components were completely separated by GC

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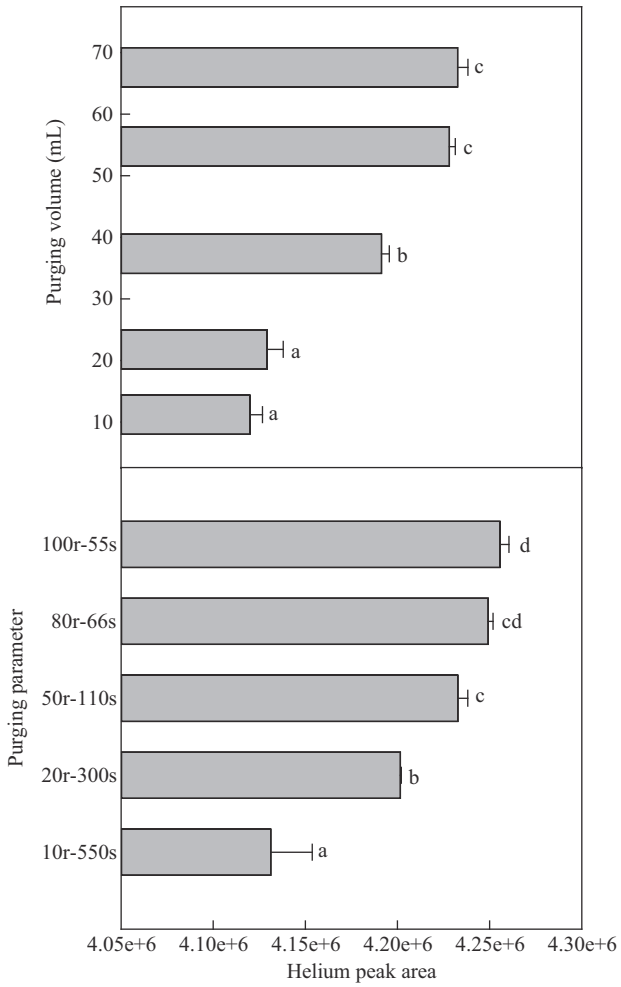

Fig. 3 – Detected peak area of helium in the sample bottle at different purging volume and different purging parameters. Horizontal bars represent standard deviations. Bars with different letters (a–d) are significantly different ($p < 0.05$), the bars with the same letters are not significantly different ($p > 0.05$).

Table 3 – Analytical characteristics of air nitrogen, nitrogen gas mixture, helium, oxygen, and nitrous gas determination by the new method.

Gas	R^2	LOD [a]	RSD [b]	Recovery [c]
Nitrogen gas	0.9997	0.02%	0.29%	100.28%
He	–	0.59%	0.60%	98.68%
Air (N_2)	–	–	0.42%	99.82%
O_2	0.9975	1.02%	0.24%	99.55%
CH_4	0.9999	0.40%	0.49%	98.50%
CO_2	0.9998	0.02%	0.71%	89.50%
NOx	0.9991	0.01 μL/L	0.94%	99.30%

[a] LOD = 3 S/N ratio, S/N is evaluated using the lowest-content detected peak. LOD = limit of detection; S/N = signal/noise.
[b] Peristaltic pump speed of 100 r/min, purging duration of 55 sec; RSD: relative standard deviation, $n = 4$.
[c] Recovery is the ratio of detected peak areas of the new method to the standard method.

following the BFW device method (Fig. 4). The results supported a number of studies reporting that N_2 was a significant component of ebullitions (Gao et al., 2012; Hwang and Hanaki, 2000; Magalhães et al., 2005) from waters. The N_2 concentration of ebullition was important to estimate the rate of denitrification involved in the biogeochemical cycling in aquatic ecosystems (Hamersley and Howes, 2005; James et al., 2011).

The gas volumes of ebullition in this study, exceeding 60 mL (Table 2) collected in a few hours, were sufficient for the GC determination and were not a problem in previous studies from March to November (Gao et al., 2012), but almost zero volume was collected in December, January and February (data not published). The requirement of 60-mL purging volume was larger than that required in the syringe injection method. This was the fractional cost of semi-automation and convenience for the integrated sampling-measuring-purging-injecting BFW method. The 60 mL volume of N_2 corresponds to 2.68 mmol

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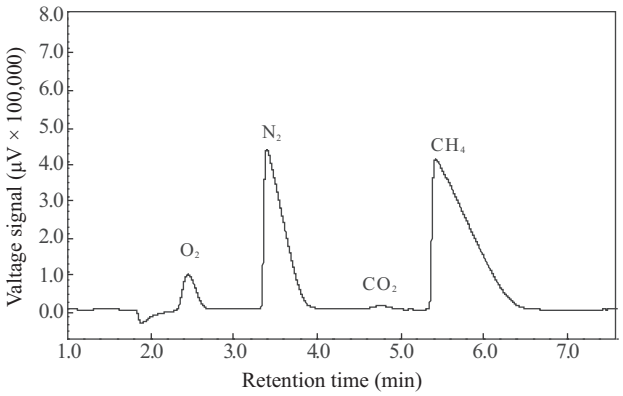

Fig. 4 – Chromatograms of real gas samples from 50 mm above sediment.

nitrogen at STP, which can be easily achieved in most aquatic ecosystems at denitrification rates ranging from 3.43 mmol/(m^2·day) to 198.43 mmol/(m^2·day) in spring, summer and autumn (Gao et al., 2013). For very small quantity gas samples, the syringe injection method is still recommended. To further reduce the requirement of purging volume, replacing the tube from the peristaltic pump to the sampling port of GC is necessary and feasible. However, this is beyond the scope of this paper.

2.5. *Comparison with other methods*

The vacuum bag (VB) and vacuum vial (VV) are common gas sampling devices using syringes and needles for sampling. The BFW device uses continuous tube connections from gas sources to sampling bottle and then to the GC. The variation and stability of different sampling devices were assessed with the indices of the average relative peak areas (ARPA, %) for N_2 at the different storage times (1, 2, 3, 5, 7 days) compared to ARPA at day 0 at 15°C. In the whole experiment period, the sample bottles and PTFE/silicon stoppers/septa used for different methods were not changed. Data indicated that the ARPA of the BFW device ranged from 99.5% to 108.5%. By contrast, the ARPA of VB and VV ranged from 123.8% to 161.1% and from 119.3% to 217.7% respectively (Fig. 5) being much higher than that of the BFW device. Furthermore, the recoveries and RSD for N_2 of the three sampling devices clearly indicated that the variation of the BFW device was the lowest, which is a desirable feature in research on the nitrogen cycle and dynamics.

Furthermore, the BFW device could provide direct measurement of gas volume, direct transportation, purging and injection of gas samples in the processes of nitrogen research, accurate and precise measurements of the individual gases (N_2, O_2, CH_4 and N_2O), and is reusable.

3. Conclusions

Through optimizing the BFW method for direct injection to a gas chromatograph (GC), high recovery of N_2, *in situ* collection and subsequent volume measurement and analysis of N_2 in gas samples were achieved. The new BFW method obtained a

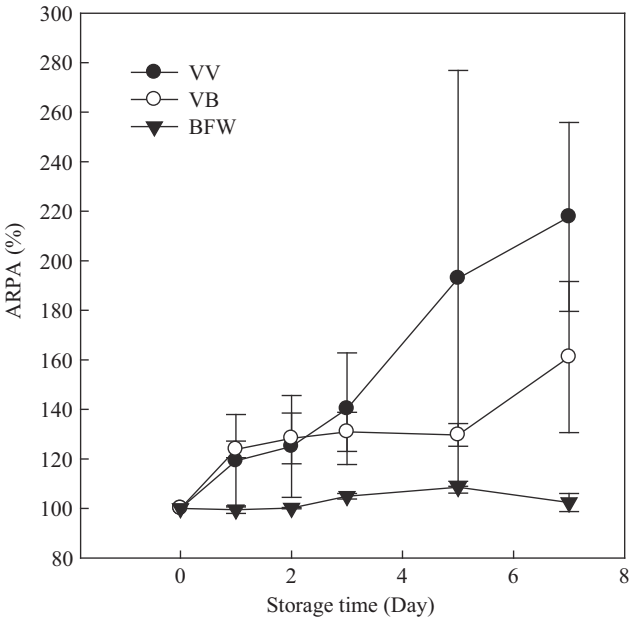

Fig. 5 – Comparison of average relative peak area (ARPA) of sampling devices of vacuum bag (VB), and vacuum vial (VV) and bottle full of water (BFW).

recovery rate of 100.28% for N_2, low indices (99.5%–108.5%) of ARPA percentage with a continuous increase of storage time up to 7 days and integrated multiple gas component (O_2, CH_4 and N_2O) analysis, and was reliable, convenient and stable in collecting, reserving and analyzing N_2 samples. It is an alternative method to be used for research on N_2 derived from biological processes in waters.

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