

Research Article

Dialysis Pretreatment for Dissolved Organic Nitrogen Analysis in Freshwaters

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Total dissolved nitrogen (TDN), including dissolved inorganic nitrogen (DIN) and dissolved organic nitrogen (DON), plays an important role in numerous environmental processes, such as nutrient cycling and lake and estuary eutrophication. The impact of DIN on environmental processes has been extensively studied. However, the understanding on DON in the environment is largely unknown, as there is no direct method for DON measurement. In practice, DON was determined by subtracting DIN from TDN. Large measurement errors could be induced when DIN becomes dominant in sample. This study was to investigate the impact of two pretreatments of dialysis, dialysis against Millipore water and dialysis against phosphate buffer (4.7 mM H₃PO₄-5.3 mM KH₂PO₄, pH 2.2) using a cellulose ester (CE) membrane (100–500 Da molecular weight cutoff), on DON measurement in a broad DIN range from various aquatic ecosystems. Results showed that the removal of NH₄-N and NO₃-N by both dialysis approaches was highly effective (>80%), but the DON loss by the former was significantly lower than by the latter. This study demonstrated that dialysis against Millipore water with the membrane would be more effective as a sample pretreatment for DIN removal, leading to a reliable and accurate DON measurement.

1. Introduction

Total dissolved nitrogen (TDN) in surface water has been increasing globally, primarily due to agriculture-related activities and inappropriate discharge of wastewater [1]. Nitrogen (N) in aquatic system exists in different forms, reflecting complex environmental and geochemical processes. TDN includes dissolved organic nitrogen (DON, e.g., protein and amino sugar) and dissolved inorganic nitrogen (DIN, e.g., nitrate, nitrite, and ammonia). Although the N-forms are interconvertible by microorganisms, DON is often a dominant N-form in aquatic systems, accounting for >50% of TDN [2–4]. Organic nitrogen is composed of >90% of total nitrogen in most soils [5], which could have a significant impact on DON composition in aquatic system due to surface runoff. DON has accounted for ~70% of the N transported by rivers to coastal waters (10¹² g yr⁻¹) [6], in which a large portion is from anthropogenic activities [7–9]. DON is an

important source of nutrients capable of stimulating algal growth, leading to eutrophication of surface waters [10, 11]. Many components in DON pool play an active role in providing N nutrition to phytoplankton and bacteria, thus affecting the DON components have been also linked to the formation of toxic disinfection byproducts [DBPs, e.g., N-nitrosodimethylamine (NDMA)] during drinking water disinfection process [13–16].

Understanding the structure and chemical properties of DON is critical for DON control and watershed management. However, the occurrence, chemical structure, and transport of DON in terrestrial environment is often limited due to the lack of DON characterization method. Currently, there is no direct method available for DON quantification [2, 17, 18], and DON characterization is often time-consuming at a high cost [19, 20]. A practical method for DON estimation is to subtract DIN from TDN [13]. High-temperature catalytic oxidation (HTCO) and alkaline persulfate oxidation are

two methods commonly used for TDN measurement. In comparison, HTO method is much easier to be implemented with commercially available instruments and capable of measuring both total organic carbon (TOC) and total nitrogen (TN) simultaneously. Hach colorimetry is one of the routine methods used for $\text{NH}_3\text{-N}$ quantification. The concentrations of $\text{NO}_3\text{-N}$ and $\text{NO}_2\text{-N}$ are usually determined by the ultraviolet-visible spectrophotometric methods [21]. Nevertheless, if DIN species are dominant in TDN, large errors could be induced for DON quantification, as reported in several studies (e.g., [13, 17, 18]). It was also found that estimated DON could become negative in contaminated water samples with extremely high DIN, for example, landfill leachates (personal communication).

A common practice to reduce the DON measurement error in water samples containing high DIN is to lower DIN concentration through sample pretreatment processes [22, 23]. Lee and Westerhoff [24] used a dialysis against deionized water with a cellulose ester (CE) dialysis membrane of 0–100 Da molecular weight cutoff (MWCO) and reported 70–80% removal efficiency of ammonium in 24 hrs. Vandenburg et al. [25] used the same CE membrane but dialyzed against $\text{H}_3\text{PO}_4\text{-KH}_2\text{PO}_4$ buffer solution (pH 2.2) and found 83–96% removal efficiency of ammonium ions. Xu et al. [26] tested a nanofiltration (NF) pretreatment by selectively concentrating DON and filtering DIN out to reduce the DIN/TDN ratio. The rejection efficiency of ammonia by three commercial membranes (NF90, NF270, and HL) was 26%, 28%, and 23%, respectively. Yu et al. [17] also used nanofiltration as a pretreatment for DON characterization in surface waters and showed 30.7% and 55.9% removal efficiency of $\text{NH}_3\text{-N}$ by two NF membranes (NF90, NF270), respectively.

Dialysis is a chemical process of separating molecules or ions in solution by diffusion through a semipermeable membrane. The acceptor solution is important for optimizing the separation process, because the solution pH determines the surface charge of the membrane that has a significant impact on ion passage across membrane. This study aimed at developing a practical dialysis pretreatments for more reliable, accurate DON quantification in freshwater samples containing a broad DIN range. Two tested pretreatment methods for dialysis included dialysis against Millipore water and dialysis against $\text{H}_3\text{PO}_4\text{-KH}_2\text{PO}_4$ buffer (4.7 mM H_3PO_4 -5.3 mM KH_2PO_4 , pH 2.2). A CE dialysis membrane with 100–500 Da MWCO and the isoelectric point (IEP) of pH 2.2 (Spectra Por, Spectrum Laboratories Inc., CA) was selected for this study. Our membrane selection was based on the hypothesis that larger pore size would help increase DIN removal efficiency and reduce dialysis time with a reasonable DON loss. In addition, the smaller pore size membrane used by Lee and Westerhoff [24] and Vandenburg et al. [25] was no longer commercially available.

2. Materials and Methods

2.1. Sample Collection. Water samples were collected at a three-month interval from a mid-Missouri watershed during

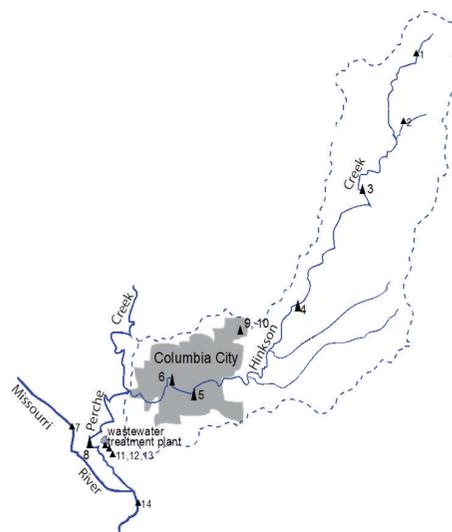


FIGURE 1: Map of the sampling sites in a mid-Missouri watershed near Columbia, MO, USA (dashed line encloses Hinkson Creek watershed and solid triangles indicate the sampling locations. Site 1 at cropland, Site 2 at grass/forest ecosystems, Site 3 at cropland/grass/forest mixed ecosystems, Sites 4–6 at urban area, Sites 7, 8, and 14 at Missouri River, Sites 9 and 10 at landfill, and Sites 11–13 at constructed wetland).

the period of February to June in 2013. The sampling sites represented a variety of ecosystems, including Hinkson Creek, Missouri River, wastewater treatment plant, and landfill sites (Figure 1). Detailed description of the sampling sites can be found in [27], including land use and flow rates of Missouri River and Hinkson Creek. Specifically, Site 1 represented crop-dominated land with 49% cropland and 13% grassland. Site 2 represented grass/forest land with 38% forest and 33% grassland. Site 5 was a United States Geological Survey (USGS) monitoring site in Hinkson Creek. Site 7 was on Missouri River, which was selected as the natural background for surface water. The water samples collected at Sites 1, 2, 5, and 7 were considered as being affected by non-point-source pollution. Sites 9 and 10 were at the city landfill with a fourteen-year-old landfill cell and a five-year-old landfill cell, respectively. Sites 11, 12, and 13 were wastewater influent, constructed wetland influent, and effluent within the wastewater treatment plant, respectively. The wastewater treatment plant utilizes activated sludge for the removal of dissolved organic contaminants and the effluent from the plant was further polished by the constructed wetland. Sites 9–13 were considered as being influenced by point-source pollution. Selected water quality parameters, including pH, dissolved oxygen, and electrical conductivity (data not shown), were measured on site when the samples were collected. The collected samples were transported in an iced cooler to laboratory, then filtered through 0.45 μm nylon membrane with polypropylene syringe filters (Fisher brand, Gel Healthcare UK Limited), and stored in refrigerator prior to chemical analysis.

2.2. Procedures of Sample Pretreatment. The procedures of the two pretreatments of dialysis for DON analysis were

similar to those described by Lee and Westerhoff [24] and Vandenbruwane et al. [25], but a larger pore size (MWCO 100–500 Da) CE dialysis membrane was selected for a quick DIN removal in the samples. The first dialysis pretreatment was conducted against Millipore water (18.2 M Ω -cm) while the second dialysis was against phosphate buffer (4.7 mM H₃PO₄-5.3 mM KH₂PO₄, pH 2.2). Both pretreatments were conducted in dark at 50:1 volume ratio of acceptor solution to sample. The pH of the acceptor solution was the same as the membrane IEP (pH = 2.2). The acceptor solution was replaced completely every eight hours during 48 hr period. All experiments were duplicated, with total of sixty samples analyzed.

2.3. TDN, DOC, and DIN Analyses. TDN and DOC (dissolved organic carbon) were determined by Shimadzu TOC-V analyzer coupled with TN-module (Shimadzu Co.), with TDN and DOC detection limits of 20 $\mu\text{g L}^{-1}$ and 50 $\mu\text{g L}^{-1}$, respectively. The procedure of data quality assurance and control (QA/QC) included (1) 6-point calibration curve with triplicated measurements at each point and the standard deviation among measurements of <5%, (2) duplicated measurements per sample, and (3) at least one standard check per sample batch. DIN species quantified included NH₃-N, NO₃-N, and NO₂-N. The concentration of NH₃-N was determined by Hach calorimetric method (Hach Co.) with the detection limit of 10 $\mu\text{g L}^{-1}$. NO₃-N was measured by ultraviolet spectrophotometric method using a Varian Cary 50 UV-visible spectrophotometer [21] with the detection limit of 60 $\mu\text{g L}^{-1}$. NO₂-N was determined by colorimetric method [21]. Because NO₂-N in all samples was below or close to the detection limit, measured NO₂-N was not included for the calculation of DIN or DON. As DON was estimated by the difference between TDN and DIN, the DON detection limit was considered as 60 $\mu\text{g L}^{-1}$. All analytical data were represented as an average of duplicated measurements.

In addition, DOC and DON in the samples were also characterized by three-dimensional excitation-emission fluorescence (EEM), nuclear magnetic resonance (NMR), and X-ray photo absorption spectroscopy (XPS), but data will be reported in separate manuscripts.

2.4. Data Analysis. The DON concentration as [DON] in the samples was estimated by subtracting [DIN] from [TDN], as shown in

$$[\text{DON}](n) = [\text{TDN}](n) - [\text{DIN}](n), \quad (1)$$

where [DIN](n) = [NO₃-N](n) + [NO₂-N](n) + [NH₃-N](n), $n = 0, 1$, and 2, referred to as raw water (0) and water pretreated by dialysis against Millipore water (1) and by dialysis against phosphate buffer (2), respectively. As [NO₂-N] in the samples was below or close to the detection limit, it was therefore excluded from [DIN].

To compare the removal efficiency of inorganic nitrogen by these two pretreatment methods, the removal percentage of NH₃-N and NO₃-N after the two pretreatments was calculated and defined as $\alpha(n)$ and $\beta(n)$ as shown in (2) and (3), where n was the same as those in (1). A ratio of [DIN]

reduction/[TDN] reduction was also calculated and defined as $\gamma(n)$ as shown in (4), illustrating the removal efficiency of DIN by the pretreatments:

$$\alpha(n) = \frac{([\text{NH}_3\text{-N}](0) - [\text{NH}_3\text{-N}](n))}{[\text{NH}_3\text{-N}](0)}, \quad (2)$$

$$\beta(n) = \frac{([\text{NO}_3\text{-N}](0) - [\text{NO}_3\text{-N}](n))}{[\text{NO}_3\text{-N}](0)}, \quad (3)$$

$$\gamma(n) = \frac{([\text{DIN}](0) - [\text{DIN}](n))}{([\text{TDN}](0) - [\text{TDN}](n))}. \quad (4)$$

3. Results and Discussion

3.1. N Species in the Raw Waters. The concentration of various N species in the raw water samples was listed in Table 1. The raw water samples displayed a broad range of TDN (1.27–633 mg L⁻¹), with 6 surface waters having low TDN (<5 mg L⁻¹ at Sites 1, 2, 5 and 7), 3 waste waters with medium TDN (>5 mg/L and <100 mg L⁻¹), and 6 landfill leachates containing high TDN (>100 mg L⁻¹). NH₃-N was a dominant N species in all landfill leachates. Data indicated that the determination of DON by the conventional method as described in (1) was not reliable, as illustrated in samples #8 and 9 having a negative DON value.

3.2. N Species in the Dialyzed Waters. The concentrations of various N species in the samples pretreated by dialysis against Millipore water or against phosphate buffer were shown in Figures 2 and 3, respectively. Data indicated that, after either pretreatment of dialysis, [NH₃-N], [NO₃-N], and [TDN] were all reduced. When [DIN] value was less than [TDN], [DON] was estimated and presented in Figures 2 and 3. In general, DON became the dominant nitrogen species in the samples after dialysis, and higher [NH₃-N](0), [NO₃-N](0), and [TDN](0) in raw waters, more DIN reduction by the dialysis pretreatment. For example, [TDN](0), [NH₃-N](0), and [NO₃-N](0) were 2.90, 0.35, and 1.10 mg L⁻¹, respectively, in sample #3 (raw water, Table 1). After dialysis against Millipore water, [TDN](1), [NH₃-N](1), and [NO₃-N](1) became 1.50, 0.02, and 0.11 mg L⁻¹, respectively (Figure 2). In contrast, [TDN](0), [NH₃-N](0), and [NO₃-N](0) in sample #7 (landfill leachate) were 600, 590, and 5.58 mg L⁻¹, respectively (Table 1). [TDN](1), [NH₃-N](1), and [NO₃-N](1) were reduced to only 33.4, 1.20, and 0.18 mg L⁻¹, respectively, after pretreatment (Figure 2).

3.3. DIN Removal Efficiency. Although DON could be estimated by the difference between [TDN] and [DIN] after either dialysis pretreatment, an important question that needed to be addressed is, which method is better for more accurate DON quantification? The effective pretreatments should be the one that has the highest DIN removal efficiency with minimum DON loss. To compare the DIN removal efficiency by the two pretreatments of dialysis, the removal percentages of NH₃-N ($\alpha(n)$) and NO₃-N ($\beta(n)$) and

TABLE 1: The concentrations of TDN(0), NH₃-N(0), NO₃-N(0), DIN(0), and DON(0) in the raw water samples.

Sample #	[TDN](0) mg L ⁻¹	[NH ₃ -N](0) mg L ⁻¹	[NO ₃ -N](0) mg L ⁻¹	[DIN](0) mg L ⁻¹	[DON](0) mg L ⁻¹
Sample #1 (Site 1)	3.56	0.05	1.15	1.20	2.36
Sample #2 (Site 1)	1.81	0.30	0.46	0.76	1.05
Sample #3 (Site 2)	2.90	0.35	1.10	1.45	1.45
Sample #4 (Site 2)	1.37	0.25	0.15	0.40	0.96
Sample #5 (Site 5)	1.27	0.24	0.25	0.49	0.78
Sample #6 (Site 7)	1.93	0.32	0.95	1.27	0.66
Sample #7 (Site 9)	600	590	5.58	596	4
Sample #8 (Site 9)	405	400	8.71	409	—
Sample #9 (Site 9)	633	640	8.64	649	—
Sample #10 (Site 10)	314	250	13.3	263	51
Sample #11 (Site 10)	218	210	3.44	213	5
Sample #12 (Site 10)	372	340	5.22	345	27
Sample #13 (Site 11)	41.2	30.0	8.79	38.8	2.4
Sample #14 (Site 12)	9.02	3.80	1.12	4.92	4.10
Sample #15 (Site 13)	4.99	2.21	0.27	2.48	2.51

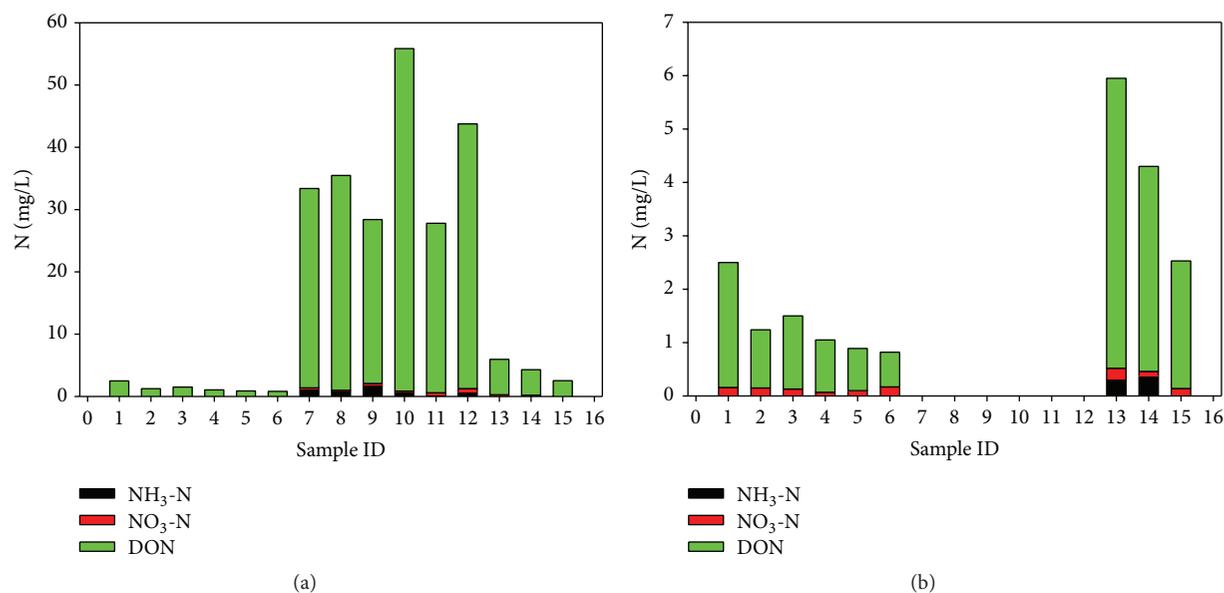


FIGURE 2: The concentrations of selected nitrogen species [NH₃-N(1), NO₃-N(1), and DON(1)] after dialysis against Millipore water. (a) The concentrations for all samples were presented and (b) only samples 1–6 and 13–15 with much lower TN were presented. Please note the difference of y-axis scale between (a) and (b).

the ratio of [DIN] removal/[TDN] removal ($\gamma(n)$) were listed in Table 2.

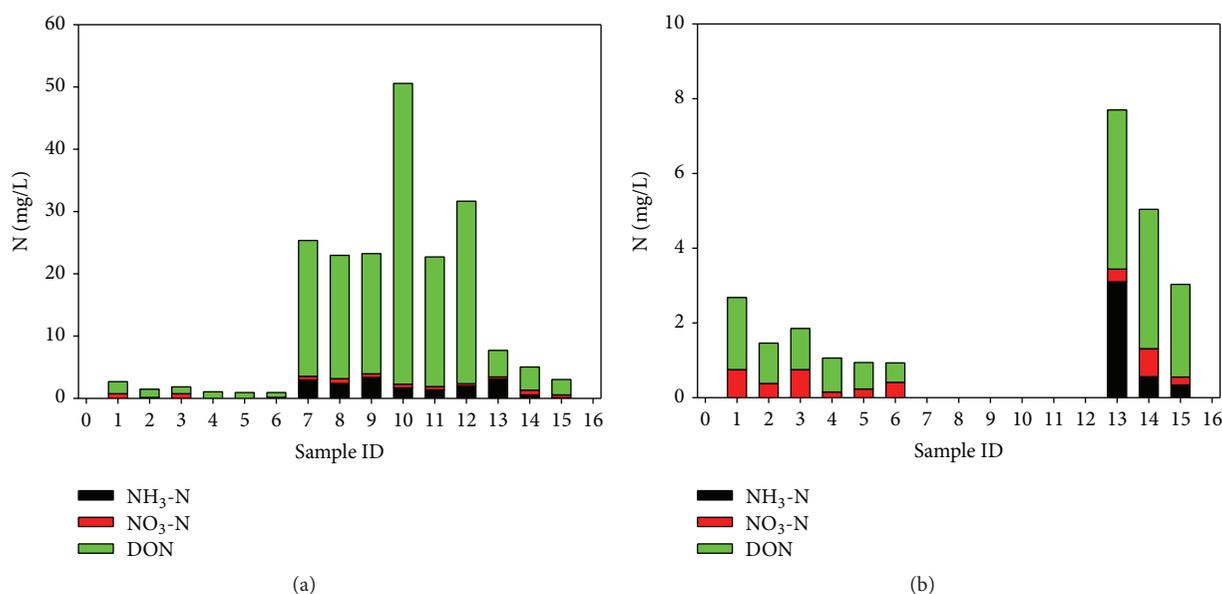
Both pretreatments of dialysis showed a high NH₃-N removal efficiency, especially in the samples with high DIN (samples #7–14). The NH₃-N removal by dialysis against Millipore water ($\alpha(1)$) appeared more effective than that by dialysis against phosphate buffer ($\alpha(2)$) with all $\alpha(1)$ values of >90%. When NH₃-N in raw water was >30 mg L⁻¹, $\alpha(1)$ values were close to 100%. The NH₃-N removal efficiency measured in our system was comparable to that reported by Vandenbruwane et al. [25] and Lee and Westerhoff [24] but

higher than that by Xu et al. [26] and Yu et al. [17]. 70–80% removal rate of NH₃-N was reported by Lee and Westerhoff [24] who used MWCO 100 Da CE membrane dialysis against deionized water. Vandenbruwane et al. [25] found that NH₃-N could be reduced by 83%–96% using MWCO 100 Da CE membrane dialysis against phosphate buffer. The removal efficiency of NH₃-N by nanofiltration was only 23 to 56% [17, 26].

There was also a significant reduction in [NO₃-N] by both dialyses. After dialysis against Millipore water, $\beta(1)$ ranged from 63% to 84% when [NO₃-N](0) was <1.0 mg L⁻¹;

TABLE 2: The removal percentages of $\text{NH}_3\text{-N}$ ($\alpha(n)$), $\text{NO}_3\text{-N}$ ($\beta(n)$), and the ratio of [DIN] removal/[TDN] removal in ($\gamma(n)$).

Sample #	Dialysis against Millipore water			Dialysis against buffer		
	$\alpha(1)$ %	$\beta(1)$ %	$\gamma(1)$ %	$\alpha(2)$ %	$\beta(2)$ %	$\gamma(2)$ %
Sample #1 (Site 1)	100	86	98	80	36	51
Sample #2 (Site 1)	93	72	107	90	24	109
Sample #3 (Site 2)	94	90	94	91	35	67
Sample #4 (Site 2)	92	67	103	88	20	81
Sample #5 (Site 5)	92	68	103	88	20	77
Sample #6 (Site 7)	94	84	99	88	61	86
Sample #7 (Site 9)	100	97	105	100	89	103
Sample #8 (Site 9)	100	98	110	99	91	106
Sample #9 (Site 9)	100	98	107	99	93	106
Sample #10 (Site 10)	100	98	102	99	96	99
Sample #11 (Site 10)	100	94	112	99	85	108
Sample #12 (Site 10)	100	90	105	99	93	101
Sample #13 (Site 11)	99	98	109	90	96	106
Sample #14 (Site 12)	90	93	95	85	33	91
Sample #15 (Site 13)	98	63	96	85	22	98

FIGURE 3: The concentrations of selected nitrogen species [$\text{NH}_3\text{-N}(2)$, $\text{NO}_3\text{-N}(2)$, and $\text{DON}(2)$] after dialysis against phosphate buffer. (a) The concentrations for all samples were presented, and (b) samples 1–6 and 13–15 with much lower TN were presented. Please note the difference of y-axis scale between (a) and (b).

however, when $[\text{NO}_3\text{-N}](0)$ was $>1.0 \text{ mg L}^{-1}$, $\beta(1)$ became $>86\%$. In comparison, $\beta(2)$ in dialysis against phosphate buffer was $<61\%$ when $[\text{NO}_3\text{-N}](0)$ was $<1.0 \text{ mg L}^{-1}$, but $\beta(2)$ significantly increased to $>89\%$ as $[\text{NO}_3\text{-N}](0)$ reached 5.0 mg L^{-1} . The $\text{NO}_3\text{-N}$ removal efficiency was measured at 80% by Lee and Westerhoff [24] and nearly 100% by Vandenbruwane et al. [25] in samples with an initial high nitrate, using MWCO 100 Da CE membrane dialysis against phosphate buffer. The removal rates of $\text{NO}_3\text{-N}$ by three

commercial nanofiltration membranes were in a range of 8.0–73.1% [17, 26].

3.4. DON Loss. The loss of small DON molecules is inevitable through membrane dialysis. To assess the applicability of the two methods of dialysis, DON loss by dialysis process was also evaluated. Theoretically, increasing MWCO from 0–100 to 100–500 might lead to increased DON loss if the DON molecular weights fall in a 100–500 range. But this might not

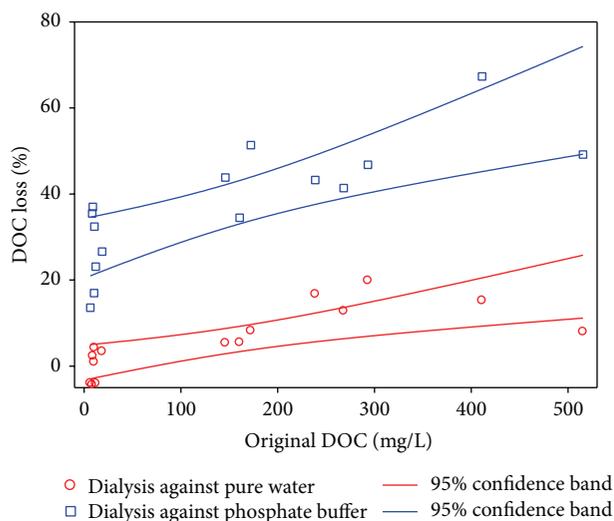


FIGURE 4: DOC loss after dialysis against Millipore water (red dots) and phosphate buffer (blue dots).

be a case based on the published date. DON in aquatic system is primarily composed of degraded amino sugars, peptides, and porphyrins [28]. Most DON molecular weights of 16 raw drinking source waters measured >1000 Da [15, 29], while proteins (such as enzymes, modified bacterial wall proteins, dissolved combined amino acids, nucleic acids, and humic-like substances) represented a dominant DON pool with larger molecular weights (usually >1 kDa) [2, 13].

DON loss by two pretreatments of dialysis could be qualitatively estimated by two parameters: the percentages of DOC loss after dialysis and $\gamma(n)$ (the ratio of [DIN] removal/[TDN] removal). The rationale to use DOC loss for estimating DON loss was based on the assumption that DON loss was related to DOC loss. When $\gamma(n)$ value approaches 1.0, DON loss is minimal. As shown in Figure 4, there was a significant difference in DOC loss between the two methods of dialysis. For dialysis with Millipore water, DOC loss was $<5.0 \pm 1.0\%$ when [DOC] was <50 mg L^{-1} , but DOC loss increased slowly as [DOC] increased and reached a maximum at $20.0 \pm 6.0\%$. On the other hand, DOC loss by the dialysis with phosphate buffer was significantly high, even at relatively low [DOC]. The loss was $>30\%$ when [DOC] was >100 mg L^{-1} , and the maximum loss could be up to $67.0 \pm 15.0\%$. Lee and Westerhoff [24] reported $16 \pm 14\%$ of DOC loss by dialysis against deionized water. Similarly, $13 \pm 9\%$ DOC loss was reported by Vandenbruwane et al. [25] for dialysis against phosphate buffer. The rejection efficiency of DOC by three commercial NF membranes was 77%, 78%, and 82%, respectively, as reported by Xu et al. [26], which could be converted as DOC loss as 23%, 22%, and 18%, correspondingly. In our dialysis system, the larger pore size of the membrane could enhance the passage of small inorganic nitrogen species; nevertheless, DOC loss by the MWCO 100–500 Da CE membrane dialysis against Millipore water was comparable with those reported above. DOC loss by dialysis against phosphate buffer appeared too high as a practical

pretreatment. In the H_3PO_4 - KH_2PO_4 buffer solution, dialysis membrane was protonated and neutral. As a result, the electrostatic repulsion between membrane and DOC was reduced, which would allow some charged DOC molecules to pass through the membrane readily, leading to increased DOC permeability. Another plausible reason for the high DOC loss could be caused by the desorption of bounded amino acids due to the replacement by phosphate and hydrogen ion, allowing amino acids to easily pass through the membrane and resulting in increased DOC loss.

The ratio of [DIN] removal/[TDN] removal ($\gamma(n)$) of the samples by dialysis pretreatments could be used to verify whether DIN was the dominant species removed. Data in Table 2 showed that all $\gamma(1)$ values were in a range of 94–112% and all $\gamma(2)$ in 51–109%, suggesting that dialysis against Millipore water was more effective for DIN removal. When [TDN] was >40 mg L^{-1} , γ value of both dialyses was close to 1.0, confirming that DIN was the dominant species removed by dialysis against Millipore water.

4. Conclusions

Two dialysis pretreatments, dialysis against Millipore water and dialysis against H_3PO_4 - KH_2PO_4 buffer, were investigated as a sample pretreatment for DON quantification in a broad DIN range (a few to a few hundred mg L^{-1}). Results indicated that both dialyses showed high efficiency for NH_3 -N removal ($>80\%$), especially with high $[\text{NH}_3\text{-N}]$ in raw water (near 100%) that often causes erroneous DON measurement. Two dialysis methods also displayed a high NO_3 -N removal efficiency, especially at high $[\text{NO}_3\text{-N}]$. Dialysis against Millipore water resulted in much lower DOC loss as compared with dialysis against phosphate buffer. This study demonstrated that dialysis against Millipore water using 100–500 Da MWCO CE membrane would be more effective for DIN removal and increase the analytical precision of DON measurement in water samples with a high DIN/TDN ratio, which would provide a valuable tool for nutrient quantification in water quality assessment, water treatment, and watershed management.

Disclaimer

The study, however, has not been subjected to the Agencies' required peer and policy review and therefore does not necessarily reflect the views of the Agencies and no official endorsement should be inferred.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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