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Application of Pretreatment Methods for Reliable Dissolved Organic Nitrogen Analysis in Water—A Review

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Dissolved organic nitrogen (DON) is ubiquitously present in the environment, and its occurrence plays important roles in nitrogen cycling and water safety issues. However, analytical techniques for DON are currently not well established. The DON data obtained from conventional methods by subtracting dissolved inorganic nitrogen (DIN, including nitrate, nitrite, and ammonia) from total dissolved nitrogen (TDN) are subject to aggregated analytical errors and therefore can be low in precision and accuracy. To minimize the subtraction-induced errors, in recent years scientists have examined several pretreatment methods to achieve either direct detection or more reliable analysis of DON. The key challenge is to maximize the extents of DIN removal and DON recovery simultaneously. This review summarizes these pretreatment methods within four categories: (1) membrane methods, (2) adsorptive methods, (3) catalysis methods, and (4) preconcentration methods. By identifying the advantages and disadvantages of each method, this review may facilitate future development of DON detection methods and make DON analysis more rapid, direct, accurate, and precise. According to available information, many studies have endorsed the dialysis method, which is thus favored, except that its processing

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time needs to be dramatically shortened. Adsorptive methods using ion exchange resins, chromatographic columns, or nonionic adsorbents have been investigated in a few cases with promising results but mixed information regarding the DON loss. Therefore, their use remains controversial and may require optimization and systematic verification in the future. In one study nanofiltration achieved substantial (69–92%) removal of DIN and certain levels (18–22%) of DON loss within 4 hr; this also should be confirmed by more studies. Electrodialysis and reverse osmosis, although not evaluated previously, are known to be robust for removing DIN species and isolating DOM (DOM); therefore, they may merit exploration as alternative methods. In contrast, catalytic reduction of DIN was proven to be inappropriate for DON analysis owing to the high level of DON loss. Lyophilization and rotary evaporation were previously assumed to preserve all solutes, but some evidences suggest that they may cause considerable changes in the DOM content and integrity. Overall, these methods have laid solid groundwork for further studies, and users need to be aware of the limits and potential interferences of these methods in practice.

KEY WORDS: nitrogen analysis, organic matter, pretreatment, separation

1. INTRODUCTION

Dissolved organic matter (DOM) is a great concern in many environmental areas. Although most attention has been paid to dissolved organic carbon (DOC), considerable concern has been recently extended to the presence of dissolved organic nitrogen (DON) in the atmosphere (Cape et al., 2011), surface water (Westerhoff and Mash, 2002; Pehlivanoglu-Mantas and Sedlak, 2006; Worsfold et al., 2008), marine water (McCarthy et al., 1998), watershed sediment (Burdige and Zheng, 1998; Aufdenkampe et al., 2001; Guldberg et al., 2002), and soil (Schulten and Schnitzer, 1997; Murphy et al., 2000; van Kessel et al., 2009). Because nitrogen is usually the limiting nutrient factor in the ocean, oceanographers have extensively studied DON (Aluwihare and Meador, 2008) to understand ecological N cycling issues, such as the uptake, release, transport, and regeneration of DON (Bronk et al., 1994). Evidence shows that the intensive use of DON by humans has accelerated the N cycling process in the environment (Canfield et al., 2010). In the field of environmental engineering, DON has been found to be linked to many pollution issues, including membrane fouling (Wang and Tang, 2011), disinfection interference (Donnermair and Blatchley, 2003), formation of nitrogenous disinfection by-products (Bronk et al., 2007; Yang et al., 2007), and support of microbial growth that may give rise to eutrophication in freshwater (Liu et al., 2011) and nitrification in water conveyance systems (Zhang et al., 2009). Therefore, a better understanding of DON in the environment and the control of DON in engineered water systems are very important.

However, current methods for DON detection in water are indirect and often unreliable. Three approaches are commonly used. The first obtains DON by subtracting ammonia from total Kjeldahl nitrogen (TKN) and is particularly used for poorly nitrified wastewater. This method is complicated in operation and has a high detection limit, as the TKN detection limit is 0.2 mg N/L or greater. In addition, TKN analysis may be subject to incomplete conversion of DON when nitrate is present (Johnes and Heathwaite, 1992; Hopkinson et al., 1993; Westerhoff and Mash, 2002; Pehlivanoglu-Mantas and Sedlak, 2006), making it unreliable for drinking water samples. The second DON measurement method is elemental analysis through which total carbon, hydrogen, nitrogen, oxygen, sulfur and/or other elements can be detected simultaneously (Worsfold et al., 2008). Inorganic nitrogen species can then be subtracted from total nitrogen. However, this method is applicable only to dry, low-ash samples, which means that a time-consuming dehydration process is required. The third method, which is probably the most commonly used approach, subtracts dissolved inorganic nitrogen (DIN, i.e., ammonia, nitrite, and nitrate) species from total dissolved nitrogen (TDN). The DIN species can be analyzed by ion chromatography (APHA et al., 1998), and TDN can be measured by oxidation methods employing persulfate, UV catalyst, microwave, or high-temperature combustion processes (Hedges et al., 1993; Bronk et al., 2000; Sharp et al., 2002). Because the process of subtracting DIN from TDN can lead to an exaggerated error of measurement, the accuracy and precision of DON analysis are sometimes low and especially poor for waters with low ratios (e.g., <60%) of DON to TDN (Lee and Westerhoff, 2004). Previous researchers raised the example that a DON data point of 0.15 mg N/L may be obtained with a huge analytical error (0.19 mg N, i.e., 126% analytical error) by subtracting a DIN of 1.85 mg N/L from a TDN of 2.0 mg N/L even though all TDN and DIN detections have only 5% analytical variance (Lee and Westerhoff, 2004). Unsurprisingly, negative DON values have been reported in many laboratories around the world (Solinger et al., 2001; Lee, 2005; Chen et al., 2011), revealing the inadequacies of current methods. Although a lot of works have been done over the past several decades to optimize the detection methods for DIN and TDN (Hedges et al., 1993; Bronk et al., 2000; Sharp et al., 2002), the comparability of DON data among laboratories has not improved significantly (Sharp et al., 2002).

Pretreatment of samples to achieve direct or more accurate detection of DON is a promising approach to minimize subtraction-induced analytical errors. Similar to the DOC measurement method, which first employs acidification and air stripping to eliminate inorganic carbon species (i.e., carbonate and bicarbonate) and then equates DOC to total dissolved carbon (TDC), an ideal pretreatment method for direct DON analysis needs to maximize DIN removal as well as DON recovery. Previously tested pretreatment methods include dialysis (Lee and Westerhoff, 2004; Vandenbruwane et al., 2007), nanofiltration (NF) (Xu et al., 2010), adsorption (Leenheer et al., 2007), ion exchange (IX) (Sattayatewa et al., 2011; Graeber et al., 2012b), catalytic reduction (Ambonguilat et al., 2006), lyophilization, and rotary evaporation (RE). These works have laid a solid foundation for future development of a direct, rapid, and accurate DON detection method with a low detection limit. However, application of these methods faces a series of problems, including slow processing efficiency, complicated operation, undesired DOM reduction, and alteration of characteristics of the organic matter, which require further improvement in the future.

This article is intended to review the factors that influence the efficiencies of these pretreatment methods, discuss the advantages and disadvantages of each method, and provide some advice that may promote further advances or even a breakthrough in future studies. The pretreatment methods are classified into four categories: (1) membrane methods (Figure 1), (2)

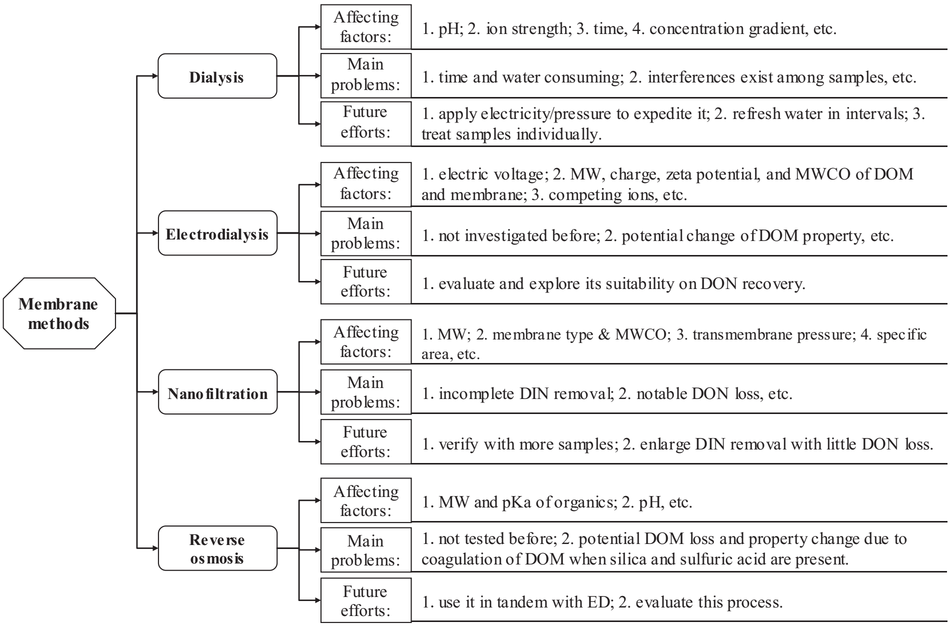

FIGURE 1. Applicability of membrane pretreatment methods for DON detection.

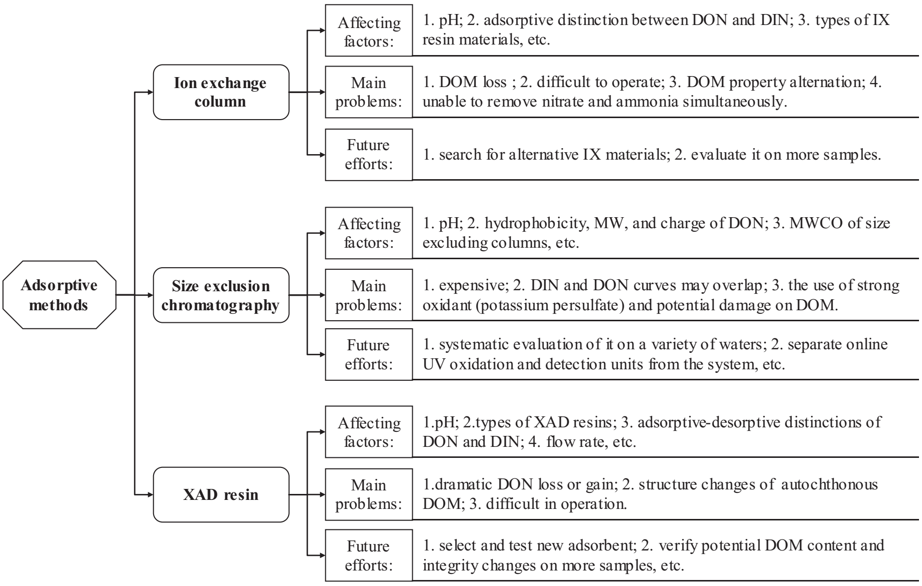

FIGURE 2. Applicability of adsorptive pretreatment methods for DON detection.

adsorptive methods (Figure 2), (3) catalysis methods, and (4) preconcentration methods (Figure 3). Two untested methods, that is, electrodialysis (ED) and reverse osmosis (RO), are discussed as well and proposed to be examined in the future because of their known capacity to separate inorganic and organic materials. In the meantime, this article summarizes some information

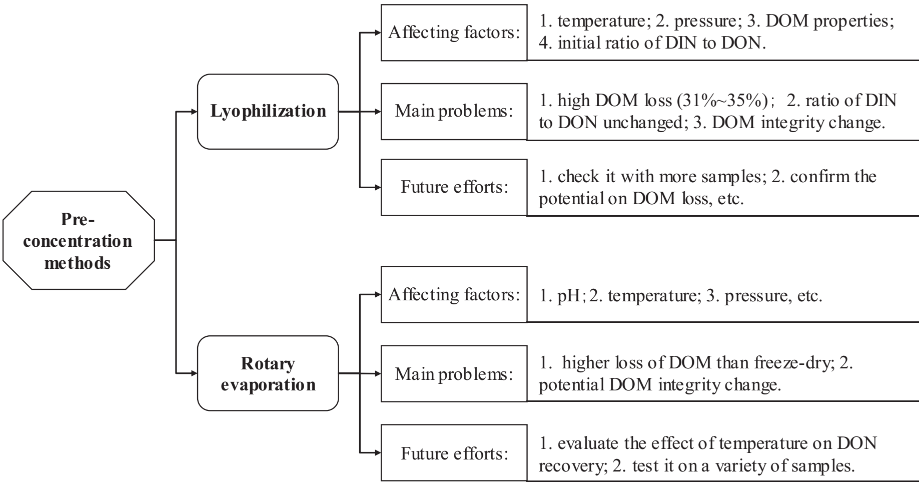

FIGURE 3. Applicability of preconcentration methods for DON detection.

on DON properties, such as molecular weight (MW), size distribution, charge intensity, and polarity, to justify the appropriateness of analytical methods.

2. METHODS

2.1. Membrane Methods

2.1.1. DIALYSIS

Dialysis is a membrane process driven by a concentration gradient to separate low MW species from large MW molecules. By carefully selecting a dialysis tube with cutoff of 100 Da, Lee and Westerhoff validated the application of dialysis process to DIN and DON separation for DON analysis (Lee and Westerhoff, 2004). Removal rates were provided for DIN species (i.e., ammonia, nitrite, and nitrate) and several representative DON compounds including urea, amino acids, peptides, and DOM isolates. Over a 24-hr course, the DIN concentrations were reduced by 70%, whereas more than 80% (average 95%) of DON concentrations (except urea) were maintained, demonstrating the ability of dialysis to lower the DIN to TDN ratio. Validation experiments carried out for both synthetic and real water samples suggested that the method is applicable to a wide range of waters. If the initial DIN/TDN percentage was less than 60%, the samples with and without pretreatment had comparable DON concentrations; but if the DIN/TDN ratio was >60%, the results had a large scatter. This threshold was confirmed by one study (Graeber et al., 2012b) but was 85% in another study (Vandenbruwane et al., 2007). Regardless of the threshold value, pretreating samples with high DIN/TDN ratios have improved the DON precision and accuracy based on these studies. Figure 1 lists the key findings.

The effects of time, ionic strength, type of solute, and the type and quantity of carrying media on the dialysis membrane permeability were evaluated (Lee, 2005; Runge et al., 2005). For a fixed ratio of membrane surface area to volume, a longer operation time can lead to higher DIN removal, reaching 99% after 5-day dialysis (Lee, 2005). Increasing the conductance (i.e., ionic strength) of the solution by adding NaCl and Na_2SO_4 can facilitate the permeation kinetics of inorganic salts; however, this is likely to cause DOM loss as a result of membrane swelling and larger pore size in response to an elevated concentration gradient (Lee, 2005). In contrast, another study showed that the effect of ionic strength is unimportant; addition of salt (5% NaCl, 6.5% $MgSO_4 \cdot 7H_2O$, and 4% $CaCl_2$) yielded no extra benefits for the dialysis kinetics (Feuerstein et al., 1997). Therefore, the effects of ionic strength require further evaluation. Increasing temperature may have little effect on the dialysis rate, as Feuerstein et al. achieved 99% removal of DIN and 95% recovery of DON for a surface water at 4°C with a dialysis period of 100 hr (1997). This rate is similar to that found in another study at ambient room temperature (Lee and Westerhoff, 2004).

Mixed results were reported regarding the relative permeation rates of ammonia and nitrate. Whereas ammonia has a lower MW and presumably a more rapid permeation rate than nitrate (Lee, 2005), it exists mostly in cationic form (i.e., NH_4^+) at ambient pH (6–8) and can be electrostatically retarded by the negatively charged membrane (Vandenbruwane et al., 2007). To counteract the retardation, Vandenbruwane et al. (2007) buffered the carrier solution to pH 2.2, which is near the isoelectric point of the dialysis membrane, and thus increasing the permeation speed of ammonia. Contrary to the membrane swelling effect in a nonbuffered sample, a considerable reduction (30.8% ± 9.8%) of water volume was observed due to the reversed ion concentration gradients between carrying water and sample, but no side effects were reported.

DOM adsorption, biodegradation, and leaching of low MW compounds can contribute to the difficulty in closing the mass balance of DON and DOC. In one early study, biodegradation of DON appeared to be a negligible cause of DON loss in samples prefiltered by a 0.7 μm glass fiber filter (Lee and Westerhoff, 2004). Adsorption of organic matter and permeation of low MW substances together accounted for up to 20% loss of DOC (Lee and Westerhoff, 2004) or 16% ± 14% loss of DOM (Vandenbruwane et al., 2007).

Maintaining the properties of the DOM during the pretreatment process may enable subsequent characterization of the treated samples. In general, dialysis will not affect DOM properties significantly, except that the small molecules (<100 Da), which are the labile fraction of DOM in water, may permeate through the dialysis membrane. An isotopic analysis confirmed that few elemental composition changes occurred before and after dialysis (Feuerstein et al., 1997).

The issues for dialysis originate mainly from the operation time and the volume of carrying water that is consumed (Figure 1). Thus far, a 5-day period is needed to achieve 99% removal of DIN and at least 1 day to decrease the DIN content by 70%. This is not very convenient for common laboratory analysis and especially inappropriate for samples with high salt content, such as marine samples, because at least 216 hr may be required to achieve relatively complete DIN removal (Vandenbruwane et al., 2007). To shorten the processing time, it may be appropriate to apply an electric force to the dialysis system. To reduce the amount of water, distilled or buffered water can be refreshed by pulse input rather than by continuous input (Vandenbruwane et al., 2007).

Another concern of the dialysis method is associated with interference between samples. To save water, early studies often dialyzed many samples in a single container (Lee and Westerhoff, 2004; Vandenbruwane et al., 2007), which may trigger migration of inorganic species from one sample to another. For example, groundwater initially containing 0.06 mg/L of ammonia was found to have a higher concentration (0.13 mg/L) after dialysis

(Vandenbruwane et al., 2007). Therefore, individual pretreatment of samples is probably a better option.

2.1.2. ELECTRODIALYSIS

ED separates organic and inorganic solutes based on the charge, MW, and electrokinetic property (i.e., zeta potential) distinctions of solutes under an external electric force. It was reported that the rate of desalination under a certain ion load is dependent on water chemistry factors (pH, conductivity), electric power (voltage, current), and the specific area of ion exchange membrane (IEM) (Koprivnjak et al., 2006). Although ED has not been applied to DON detection, it is known to eliminate nitrate faster than dialysis (Abou-Shady et al., 2012). Therefore, this article provides some sample studies that demonstrate the feasibility of the ED process for separating DIN and DON (Table 1).

As known, the performance of the ED process in organic/inorganic matter separation is dominated by two groups of factors: (1) the properties of the DOM, for example, MW, charge, and zeta potential, and (2) the properties of the IEM, for example, exchange capacity, pore size, surface charge, and type of functional group (Kim et al., 2003). The low MW, hydrophilic DOM fraction is responsible for the loss from leaching and the large MW, hydrophobic, charged DOM is responsible for the adsorptive loss during the ED process. Together, the permeated and adsorbed organics led to less than 10% loss of DOC (Kim et al., 2003). Koprivnjak et al. (2006) assessed the efficiency of ED in reducing sulfate, which is more difficult to remove than nitrate, in synthetic and real river waters. When NaOH and NaCl were occasionally added to maintain pH >6 and conductivity >1 m S/cm, a 0–12% loss of DOC (average 4%) was detected in synthetic waters along with 95% removal of sulfate within 10 hr. Because single-charged DIN ions (i.e., nitrate, nitrite, and ammonia) always move faster than polyvalent ions (e.g., sulfate), DIN loss is hypothesized to be higher than 95% under the same experimental conditions. For concentrated samples consisting of RO retentates, ED pretreatment achieved 88–94% DOC recovery (Koprivnjak et al., 2006), which was comparable to the DOM loss (less than 9.1%) reported by an early study (Lee et al., 2003a). These results hence suggest that ED is a promising approach for the separation of DON and DIN to facilitate a reliable DON analytical method (Figure 1).

The rest of this section describes the differences between DIN and DON with respect to their MW, zeta potential, and charge characteristics as found in other studies. The MW differences between DON and DIN are obvious. Whereas DIN species have MWs of less than 100 g/mol, the average MWs of the hydrophobic, transphilic, and hydrophilic fractions of DOM in surface waters were typically 1840, 1650, and 670 g/mol, respectively, with

TABLE 1. Important Literature Results for Pretreatment Efforts that Might be Used for DON Detection

Method	Condition	Effect on DIN removal	Effect on DON/DOC/DOM recovery	Effect on DON/DOC/DOM integrity	References
Conventional dialysis	4°C, up to 100 hr	99% removal	>95% DON recovery	yes in terms of elemental composition	Feuerstein et al. (1997)
	room temperature, up to 120 hr	70–80% within 24 hr and >99% within 120 hr	usually <15% loss in 120 hr	not provided	Lee and Westerhoff (2004)
	same as above	more NO_3^- removal than NH_4^+	16% ± 14% loss	minor due to leaching of small MW compounds	Vandenbruwane et al. (2007)
Buffered dialysis	set pH at 2.2 with phosphorus buffer	faster NH_4^+ removal than conventional dialysis	13% ± 9% loss	likely due to pH change	Vandenbruwane et al. (2007)
Nanofiltration	real waters, <4 hr, pH 3–6	69–92% NO_3^-, 72–77% NH_4^+	77–82% of initial DOC	83–88% of initial UVA	Xu et al. (2010)
Electrodialysis	pH >6, conductivity >1 m S/cm, current <1 A, voltage <18.7, <10 hr	95% removal of sulfate, as a replacement for DIN, in synthetic waters; 41% removal for real waters	0–12% (4% on average) loss of initial DOC for all waters	not provided	Koprivnjak et al. (2006)
	0.1 M NaCl, pH 5.5–7.0, flow rate 0.2–0.3 L/min	84–89% NaCl removal in the presence of DOM	90.9–92.5% DOC recovery	MW reduced to 1460 from initial 1570	Lee et al. (2003a)
Reverse osmosis	potable RO system, coupled with ED	0–6% loss of sulfate, 33–42% loss of chloride	average 12% loss of DOC	little when silicate and sulfate are preremoved by ED	Koprivnjak et al. (2006)
	synthetic and real waters	high recoveries of simple salts, such as NaCl	usually 90% recovery	possible if silicate and sulfate are absent	Sun et al. (1995)
	synthetic and real waters	not provided	>120% DOC for urea and histamine, implying organic releases	not provided	Chang and Wang (2013)

(Continued on next page)

TABLE 1. Important Literature Results for Pretreatment Efforts that Might be Used for DON Detection *(Continued)*

Method	Condition	Effect on DIN removal	Effect on DON/DOC/DOM recovery	Effect on DON/DOC/DOM integrity	References
Ion exchange column	set pH <2, AER	achieve <0.003 mg/L of nitrate	92–110% recovery for 17 real effluents	not provided	Sattayatewa et al. (2011)
	AER and CER	>90% removal of DINs	75% ± 13% of DON recovery	not provided	Bronk and Glibert (1991)
	AER or CER	not provided	12–32% removal for AER, 11–49% removal for CER	not provided	Pehlivanoglu-Mantas and Sedlak (2006)
	zeolite, 60 min	>90% of NH_4^+ removal	not provided	not provided	Westerhoff et al. (2006)
	AER	99% NO_3^- removal, no ammonia and nitrite removal	usually 36–74%, at most 83% DON recovery	not provided	Graeber et al. (2012a)
Size-exclusion chromatography	potassium persulfate added in mobile solution, set pH at 6.85	almost complete separation	89–108% DON recovery for model compounds, 93–101% for real samples	not provided	Graeber et al. (2012b)
	weak cation exchange column	fractionates both NO_3^- and NH_4^+	79.3–125.2% for polymer materials	not provided	Huber et al. (2011)
XAD resin fractionation	XAD-1, XAD-4, MSC-1H in sequence	not provided	overall 19% loss of DOC	likely due to the uses of acid and base	Leenheer et al. (2007)
	XAD-2	not provided	90–110% DOC recovery		Lara et al. (1997)
	DAX-8, XAD-4, MSC-1H resins in sequence	not provided	no difference for duplicates, but no recovery data	NH_4OH may react with humic substances to form polyphenolic structures	Chang and Wang (2013)

Catalytic reduction	Pd-In/Al_2O_3, Pd-Sn/Al_2O_3, and Pd/SnO_2	80% in pure water, <15% in DOM water	<15% sorption and reaction for amino acids, 23–78% in DOM water	likely	Ambonguilat et al. (2006)
	Pd-Sn/Al_2O_3	81% nitrate removal in DOM-free water, slower in DOM-containing water	10–20% DON decrease in DOM-present water	likely due to adsorption and reduction	Westerhoff et al. (2006)
Lyophilization	−53°C, 442 mbar, lyophilization followed by buffered dialysis	little	33% ± 2% loss of DOC, higher loss than no lyophilization treatment	lower solubility of DOM	Vandenbruwane et al. (2007)
	−20°C for one day	not considered	averagely <5% loss, recovery rises with rising DON content	high variability for plastic tubes	Chang and Wang (2013)
	−7°C, −50°C	little	DOC and TDP average 14% and 16% lower than the origin	0∼14% SUVA decrease	Fellman et al. (2008)
Rotary evaporation	49°C	little	higher loss than lyophilization	likely	Vandenbruwane et al. (2007)
	<40°C, 10 mm Hg	<5% DIN removal	little DON loss	not observed	Feuerstein et al. (1997)
	30°C, 40°C, 50°C	not provided	<10% loss, recovery higher at 30°C than 40°C and 50°C	loss of small molecules and volatile compounds at high temperature	Chang and Wang (2013)

AER: anion exchange resin; CER: cation exchange resin; TDP: total dissolved phosphorous.

an overall MW of 1570 g/mol (Lee et al., 2003a). After ED treatment, the average MW decreased slightly to 1460 g/mol (Lee et al., 2003a). In addition, the MWs of DON were shown to be greater than those of DOC, and large MW compounds contained more nitrogen than low MW compounds (Egeberg et al., 1999). Such information suggests that DON is probably less removable by ED process than is DOC, and therefore DON is more likely to achieve higher recovery than DOC.

As for membrane characteristics, the pore size of an IEM is usually unavailable from the manufacturer; IEMs are sometimes thought to be nonporous. However, by use of a fractional-rejection method, the apparent pore size of an anion exchange membrane (AEM) was determined to range from 100 to 200 g/mol (Kim et al., 2003). This size is close to the molecular weight cutoff (MWCO) of a dialysis membrane but smaller than that of most NF membranes. The relative size distinctions among ED membrane (100–200 g/mol), DIN (<100 g/mol), and DON (>500 g/mol) hence serve as the basis for separation of DIN and DON.

The zeta potentials of DOM molecules and IEM together control the mobility of DOM. For example, the zeta potential of an AEM was positive (+7.6 mV) and remained relatively stable over a wide pH range, 3–10 (Kim et al., 2003). In contrast, the zeta potentials of hydrophobic and transphilic DOM, which together account for ~80% of DOC, exhibited a negative electromagnetic capacity (−25 mV at pH 5.8–6.0) and a decreasing trend with increasing pH (Lee et al., 2003b). These property changes of AEM and DOM may result in more DOM adsorption on the membrane when the pH increases (Kim et al., 2003).

Charge intensity is another factor affecting the separation of DIN and DON. DOM exists mostly in neutral form and partially in acidic forms in natural water (Perdue and Ritchie, 2003). Compounds with $pK_a < 8$ typically have more carboxyl acid groups, and those with $pK_a > 8$ typically have phenolic compounds. In one study, the DOM of one water was found to contain 7.4 meq/g DOC (81.2%) of carboxyl acid and 1.7 meq/g DOC (18.8%) of phenolic compound (Kim et al., 2003). The total charge intensity (i.e., 9.1 meq/g DOC) is equivalent to only 1.3 mg/L of inorganic nitrogen when the water has 10 mg/L of DOC. The quantity is far less than DIN contents observed in many types of waters (usually range from 10 to 100 mg N/L) (Chen et al., 2011). Although an AEM has a strong exchange capacity (1.4 meq/g-dried membrane) to transfer all charged DOM species across the membrane (Lee et al., 2003a), the presence of competing ions and slow motion of organics can sometimes retard or even stop the permeation of DOM. Evidence showed that certain ions (e.g., chloride, sulfate) competed with DIN species in crossing the membrane, which is not preferred for DIN removal (Kim et al., 2003). However, it is favored if chloride competes with the charged organic acids for approaching the membrane and ultimately reduces the adsorptive loss of DOM (Kim et al., 2003). Future studies thus need to balance the needs of these contrary effects and evaluate the performance of ED on DIN and DON separation (Figure 1).

2.1.3. NANOFILTRATION

NF is another membrane approach tested once for DON analysis in water. Similar to dialysis, it isolates DON and DIN mainly on the basis of size differences. But unlike dialysis, NF imposes a pressure gradient on the system so that the kinetics of DIN migration can be significantly accelerated. Xu et al. (2010) found that three types of commercial NF membranes reduced nitrate by 69–92% and ammonia by 73–78% within just 4 hr, whereas maintained DOC recovery by 77–82% and the ultraviolet absorbance (UVA_{254}) by 83–88%. As a result, this method successfully increased the accuracy of DON detection (Table 1).

The factors affecting DIN and DON separation efficiency include but are not limited to membrane type, pH, transmembrane pressure, and ratio of sample volume to NF surface area (namely, specific area). A polyamide membrane that had the highest nominal MWCO and lowest NaCl retention capacity was the membrane with the best separation performance (i.e., maximum DIN removal and maximum DON recovery). This suggests that the MWs of the ions and MWCO of the membrane are not the only factors that affect the permeation of ions; electrical attraction and/or repulsion between the membrane and ions may also play an important role. Increasing the ratio of sample volume to surface area resulted in a reduction in DIN removal (Xu et al., 2010), which is similar to the dialysis study (Lee and Westerhoff, 2004). Because pH can control the speciation of solutes and the membrane surface charge, its effects on DON recovery appeared to be complicated. In the test, nitrate removal increased from pH 3 to pH 5 but remained constant from pH 5 to pH 10; ammonia removal averaged greater than 70% at pH <6 but decreased above pH 7. In comparison, both DOC recovery and DIN/TDN ratio increased from pH 3 to pH 7 but remained stable from pH 7 to pH 10. However, an increase in transmembrane pressure from 0.2 to 0.6 MPa did not reduce the DIN/TDN ratio (Xu et al., 2010).

With regard to potential property changes during the NF process, the specific UVA (SUVA), a parameter of DOM hydrophobicity and aromaticity, increased slightly (<10%), suggesting that the process did not alter the organic matter remarkably. A minor increase in SUVA may be attributed to the loss of hydrophilic and aliphatic compounds (Lee and Westerhoff, 2004). Interestingly, the efficiencies of DIN removal and DON recovery were lower in synthetic waters than in real samples (Xu et al., 2010); the underlying reason requires further investigation. In addition, because NF did not completely remove DIN species, it cannot be used as a direct DON detection method. Future studies should extend the operating time to try to maximize the DIN and DON separation level (Figure 1).

2.1.4. REVERSE OSMOSIS

To date, no research has evaluated the application of RO to DON analysis. However, RO is known to be robust for the separation and concentration of DOM. Therefore, this section reviews some results on the recovery of DOC, an equivalent of DON, and through which demonstrates the potential of RO for the separation of DON and DIN (Table 1).

For example, a review reported for RO a very high recovery of DOC (87.8% ± 9.8%) for 55 samples from various sources (Perdue and Ritchie, 2003). In a more recent study, RO also yielded 82–92% (averagely 88%) recovery of DOC for two natural water samples (Koprivnjak et al., 2006). However, other research indicated that approximately 34% of the DOC was lost during the RO process (Crum et al., 1996). The substantial removal of DOC was attributed to the recycling process, during which some of the DOM is either eluted from permeates or adsorbed on the membrane (Perdue and Ritchie, 2003). The extent of concentration corresponded well with the removal rates of DOC and DIN (Sun et al., 1995). Therefore, it probably would be challenging to recover DON and eliminate DIN simultaneously using this method.

The performance of RO is determined by the external pressure, rather than concentration gradient, and by the characteristics of the DOM. Through early studies, the effects of MW and pressure on the treatment efficiency of some DOM species were evaluated. The rejection rate of low MW organic acids was found to be associated with the pH value of the target solution. Using acetic acid ($pKa = 4.74$) as an example, the recovery of DOC was approximately 80% at pH 4 but 96% or greater at pH 6 (Sun et al., 1995). In contrast, increasing the pressure from 130 to 200 psi affected the recovery of DOC very little (Sun et al., 1995). With regard to DOM integrity, early studies indicated that RO can preserve the size, polarity, charge density, and isoelectric point (Kilduff et al. 2004) of DOM and maintain its reactivity with copper and zinc (De Schamphelaere et al., 2005), formation potential of disinfection by-products (Kitis et al., 2001), and UV/visible absorptivity (Gjessing et al., 1998). However, when ions such as calcium, aluminum, sulfate, and silicate are present and propagated during the RO process, condensation and/or coagulation of DOM may occur and cause changes in the DOM characteristics (Sun et al., 1995; Maurice et al., 2002). For instance, RO treatment of samples with high amounts of silicate and sulfate yielded an RO isolate with high ash content (Maurice et al., 2002). To prevent such undesired effects, removal of ions by IEM prior to RO treatment was recommended; moreover, use of an H^+-bonding cation exchange membrane was not recommended to avoid decreasing the pH and the subsequent side effects (Sun et al., 1995).

Figure 1 and Table 1 summarize the features of the membrane processes described above. In a study of coupled ED and RO, DOC loss from ED was estimated to be 4% as opposed to 12% from RO (Koprivnjak et al., 2006), which indicates the higher DOM recovery efficiency of RO. Compared to NF, however, RO appeared to be a better alternative (Siddiqui et al., 2000) because RO membranes usually have a smaller pore size (or MWCO) than NF membranes (Perdue and Ritchie, 2003).

Unlike in industrial applications, the issue of membrane fouling is not as great a concern for laboratory-scale devices in terms of the cost burden. However, understanding the causes of fouling and its effects on DOM loss is equally important for NF, RO, ED, and dialysis prior to the development of a direct DON detection method.

2.2. Adsorptive Methods

2.2.1. ION EXCHANGE COLUMN

Ion exchange (IX) is a process that removes undesired ions by replacing them with ions that are initially bonded to the IX resins. Its application to DON detection has been evaluated in a few studies (Table 1).

For a well-nitrified wastewater that contains little ammonia, a more accurate measurement of DON was achieved by removing nitrate completely from the system with an anion exchange resin (Crumpton et al., 1992). In another study aiming to detect low levels of nitrogen and phosphorus in 17 real wastewater effluents, Sattayatewa et al. (2011) examined the suitability of IX resins for direct DON analysis using several model DON compounds including urea, EDTA, and amino acids. They reported almost complete nitrate removal, lowering the nitrate to below the detection limit (0.003 mg N/L) and a DON recovery of 92–110%. As a result, the DON concentrations were well calibrated (linear regression $R^2 = 0.99$) over a wide range of concentration, from 0.05 to 3 mg N/L. However, the data without IX pretreatment (Y-axis) and with IX pretreatment (X-axis) were correlated as equation of $Y = 0.88X + 0.01$. It appeared that the data with pretreatment were on average 12% higher than those without pretreatment, which is probably attributed to either an underestimation of DON in nonpretreated samples or an unexpected release of DON from IX resins (Kemper et al., 2008; Flowers and Singer, 2013). Meanwhile, the article did not provide the DIN/TDN ratios of the tested samples, making it hard to evaluate its suitability on DIN-enriched samples. Alternatively, one study (Westerhoff et al., 2006) evaluated zeolite as an adsorbent to remove ammonia (>90%) within a short period of time (60 min), a technique that may be applicable to poorly nitrified wastewater samples. This technique has yet been applied to pretreat samples for DON analysis.

Another major problem with the IX method is the adsorptive loss of DOC (Figure 2). Graeber et al. (2012a) compared the performance of dialysis and IX resin pretreatments in DON analysis and found that the IX resins retained 36–74% of the DOC and sometimes up to 83% of the DON. In addition, Bronk and Glibert (1991), who used an ion retardation column to remove nitrate prior to DON analysis, obtained only 75% ± 13% DON recovery. These results are probably not case-specific, as a review of the literature that summarized the DOM adsorption abilities of IX resins found DOC removal ranging from 12% to 32% for three types of anion exchange resins and 11% to 49% for three types of cation exchange resins (Pehlivanoglu-Mantas and Sedlak, 2006). In general, on the one hand, DOC from target water may adsorb onto IX leading to DOC loss; but on the other hand, IX resins may release organic materials during the elution process leading to undesired DOC gain, which is problematic too.

Other drawbacks of the use of IX resin for DON detection stem from its operational difficulty and inadequate treatment of samples with various types of N species (Figure 1). For example, to minimize contamination of subsequent samples, adsorbed DIN needs to be completely desorbed from IX resins. Anion exchange resin cannot remove positively charged species, that is, ammonia, whereas cation exchange resin is not designed to remove negatively charged compounds, that is, nitrate and nitrite. Thus, it is a challenge if both ammonia and nitrate are present in water. In addition, to avoid DON adsorption and bicarbonate interference, samples need to be acidified to pH <2 (Sattayatewa et al., 2011). This approach risks altering DOM properties (Sattayatewa et al., 2011) because humic acid is operationally defined as the soluble organic fraction above pH 2 (Matilainen et al., 2011).

2.2.2. SIZE EXCLUSION CHROMATOGRAPHY

Size-exclusion chromatography (SEC), also known as gel permeation chromatography, is frequently used for advanced DOM characterization (Worsfold et al., 2008) and designed to separate molecules according to their size and dispersivity. Because DIN species are smaller and more hydrophilic than DON, the N compounds can be separated into several fractions, including large MW humic substances, biopolymers, building blocks, low MW acids and neutrals, and hydrophobic organic carbon (Huber et al., 2011). The chromatographic columns tested were made of polymethacrylate and coated with weak cation exchange resin that allows rapid evolution of hydrophilic, high MW (10 kDa or higher) substances first, followed by DOM with MW ranging from 0.1 to 10 kDa, and then nitrate and ammonia. The mobile phase was a phosphorus buffer with pH 6.85, and the detector was equipped with an ultraviolet (UV) reactor to convert nitrogen compounds to nitrate that can be detected by an inline UV detector (Graeber et al., 2012b). In addition to separating DIN and DON, the SEC method generates chromatograms of DON that provide extra information toward understanding DON characteristics. The method is more rapid than dialysis and to some extent superior to an anion exchange column because it can differentiate both ammonia and nitrate from DON in just one run (Huber et al., 2011). Another study used similar equipments with a silica-based column and $NaClO_4$ as the mobile phase to characterize DOC and DON for DOM isolates at a buffer pH of 6.5 (Egeberg et al., 1999); however, this study did not try to distinguish DIN from DON.

With regard to DON preservation, one study, within just 2.5 hr, achieved 89–107.9% recoveries of DON for seven standard compounds that were dosed with varying amounts of DIN and 93–101% recoveries for two natural samples with varying DIN to TDN ratios (Graeber et al., 2012b). Similarly, only two of 10 samples reported unexpected nitrogen recoveries in another study (Huber et al., 2011). Of all model compounds (i.e., L-tyrosine, imidazole, nicotinic acid, glycine, EDTA, and urea), only urea has indistinguishable retention time with DIN species (Graeber et al., 2012b). Because the fraction of urea in drinking water is usually low in concentration (<1% of DON) (Dotson and Westerhoff, 2009), this may not significantly affect the accuracy of the method for most of drinking water samples.

Although successful, this approach requires an expensive instrument equipped with inline UV oxidation and detection systems. The system may exclude the UV oxidation and detection units to lower the cost yet achieve the simple goal of DIN and DON separation. Because the UV oxidation method has sometimes been reported to be less efficient for oxidizing DON than the combustion method or persulfate oxidation method (Bronk et al., 2000), such a modification may lead to a more accurate DON analysis. The molecular size calibration of SEC must employ standard materials, and a program capable of integrating a wide range of DON chromatograms is also needed. In addition, concerns about if and how much DOM may be adsorbed during SEC operation need to be addressed (Huber et al., 2011). Even though it has been reported that only a small fraction ($<2\ \mu$M) of DON was lost in the column effluent (Hopkinson et al., 1993), more evidence is needed to enable a systematic verification of various types of waters. An additional concern is that a strong oxidant, either potassium persulfate (Graeber et al., 2012b) or sodium perchlorate (Egeberg et al., 1999), at a high concentration (500 mg/L) was applied to the mobile phase. The potential impact of these oxidants on DOM integrity needs to be verified. Overall, because the performance of SEC is determined by many factors (Figure 2), a proper design of pH, ionic strength, temperature, cell pressure, and selection of column and mobile phase may merit further refinements, especially for samples with very high nitrate content (e.g., >10 mg/L N).

2.2.3. XAD Resin Fractionation

DOM is often isolated and fractionated to promote understanding of its composition and characteristics. XAD resin fractionation is an operationally defined method based on the adsorption and desorption ability of DOM. A series of membrane and nonionic macroporous resins, such as XAD-8 and XAD-4 (Maurice et al., 2002), were used to isolate DOM into several categories: (1) colloid; (2) hydrophobic acid, neutral, base; (3) hydrophilic acid, neutral, base; and (4) transphilic substances. An exemplary DON characterization effort was conducted by Leenheer et al. (2007), who employed a sequence of RE, dialysis, adsorption, desorption, and lyophilization processes to obtain DON isolates (Table 1). The first isolate, which was retained by a dialysis membrane (using a cellulose membrane with 3500 Da cutoff), was termed colloids. The permeated DOM then flowed through three types of XAD resins in sequence. The retentates of the XAD-1 resin were the hydrophobic items, including acid, neutral, base, and amino acid. The fraction that permeated the XAD-1 resin but was retained by the XAD-4 resin was deemed transphilic items; the compounds that permeated the XAD-1 and XAD-4 resins but were screened by a cation exchange resin (MSC-1H) were labeled the hydrophilic bases; and what remained after all these processes was considered a combination of hydrophilic acid, neutral, and inorganic salts, including DINs.

Early research showed that the DOM recovery by this method was not very high (Buffle, 1988; Perdue and Ritchie, 2003). Leenheer et al. (2007) obtained similar results, although they collected some highly concentrated N-containing isolates for characterization. A mass balance calculation of the organic matter recovery identified a moderate (14–22%) loss of DOC in natural water and wastewater samples but considerable DOC gains (19–56%) for samples originating from algae and bacteria. The DON recovery was also unexpected, as it was as low as 16% and as high as 214% (Leenheer et al., 2007). The reason for this uncertainty is unknown but presumably originates from the concentration during lyophilization or from the potential adsorption or release of organic matter from the resins. In a similar effort, Chang and Wang modified and validated a DON fractionation method using DAX-8, XAD-4, and MSC-1H resins (Chang and Wang, 2013). The results were very promising because the total DOM loss did not exceed 10%. The minor statistical differences in DON compositions between duplicates illustrate the precision of the method (Chang and Wang, 2013).

In addition to the potential DOM loss, there were concerns regarding potential DOM property alteration owing to the adsorption–desorption process (Peuravuori and Pihlaja, 1997). Some evidence indicates that a pH decrease can result in significant DOM property changes, such as ester hydrolysis (Maurice et al., 2002) or structural changes in autochthonous DOM (Schwede-Thomas et al., 2005). Thus, although the DON content may not be affected, the use of isolates for characterization should be considered with caution.

Future efforts should search for an appropriate adsorbent and verify it in diverse source waters (Figure 2). For example, Lara et al. (1997) examined DOM concentration changes before and after use of another adsorbent, XAD-2, and a DOC recovery of 90–110% was reported. In addition to XAD resins, many types of adsorbents are available for nitrate and ammonia removal (Bhatnagar and Sillanpää, 2011). Future studies may investigate their adsorptive potentials on DON; the candidate(s) with little adsorption of DON and strong treatability on DIN can be selected later for sample pretreatment.

Only a few studies have compared the pros and cons of membrane and adsorptive methods. Relative to the XAD-resin adsorptive method, RO appeared to offer a higher yield of a broad range of DOM and is not specific to humic substances (Maurice et al., 2002). Dialysis also exhibited better DON accuracy than the IX resin method (Graeber et al., 2012a). These results, although inconclusive, suggest that both methods have raised great interest and research should continue in the future.

2.3. Catalytic Reduction

Catalytic reduction is a chemical approach to remove DIN and lower the DIN/TDN ratio in water. Ambonguilat et al. (2006) investigated three types of palladium (Pd)-based bimetallic catalysts to reduce nitrate, and another study confirmed the inadequacy of the catalysis method (Westerhoff et al., 2006). In general, catalysis was effective in reducing nitrate significantly without decreasing DON (i.e., model amino acids) (<15%) in the absence of the DOM, but this reaction was dramatically deactivated in the presence of DOM, which is a common condition in practice. For example, when DOM was present, DOC and DON reductions were only 23–78%; nitrate was transformed into ammonia rather than N_2; and the reaction rate became much slower (Ambonguilat et al., 2006). In addition, both sulfate and sulfur-containing amino acids exhibited higher reactivity than nitrate and sulfur-free amino acids, thus competing for reaction with the catalyst (Ambonguilat et al., 2006). These results thus illustrate the inability of these catalysts to separate DIN from DON (Table 1). Although other types of metal-based catalysts (e.g., platinum, the alloys used to convert nitrate and nitrite to nitrous oxide (NO) before DON analysis) may be chosen for investigation in the future (Liou et al., 2012), catalysis is not commonly used now, implying that it probably requires a more systematic evaluation.

2.4. Preconcentration Methods

2.4.1. LYOPHILIZATION

Sample preconcentration is a process designed to magnify chemical concentration to make it easy to be detected. Because the analytical errors of

DIN and TDN can be significantly lowered (e.g., from 5% to 1%) at elevated concentrations, preconcentration process may enable more precise and accurate analysis of DON. DON is usually present at below the mg/L level in freshwaters (Lee et al., 2006; Dotson and Westerhoff, 2009; Chen et al., 2011), so preconcentration prior to detection is often necessary. Some methods, such as RO and NF, can concentrate and separate samples concurrently, but lyophilization and RE are intended for sample concentration only. This section reviews the influence of these two processes on quantitative and qualitative changes in organic matter.

Lyophilization, or freeze-drying, is a dehydration process that converts water from the solid state into the vapor state without going through the liquid state. The process is often implemented at below-melting-point temperature and low-pressure conditions, and it is usually designed for long-term sample storage. In this process, TDN and DIN concentrations are expected to be dramatically enlarged such that they can be analyzed with little analytical error (Figure 3) and hereby lead to more accurate DON analysis even though the process of subtraction of DIN from TDN is inevitable.

Freezing samples is known to be effective in preserving DIN species (Bachmann and Canfield, 1996; Kotlash and Chessman, 1998) and occasionally DON as well (Chang and Wang, 2013). Some researchers have demonstrated DON recoveries close to 100% and independent of the type of container (i.e., polyethene bottle, centrifuge tube, and glass bottle) and initial DON concentration (Chang and Wang, 2013). However, a study conducted by Fellman et al. showed that the amounts of DOC and total dissolved phosphorus (TDP) were on average 14% (2.7 mg C/L) and 16% (approximately 4.4 μg P/L) lower than the original contents after freezing for just one week. The tests were carried out in two freezing conditions, a standard freezer (−7°C) and a flash freezer (−50°C), and 10 samples were collected from streams in Alaska, USA, for freezing experiment (Fellman et al., 2008). The organic matter losses were thought to result from abiotic formation of brown particles by humic substances because they paralleled a 0–14% SUVA decrease (Fellman et al., 2008). Occasionally, DOM loss has also been attributed to volatilization (Giesy and Briese, 1978). Unlike DOC and TDP, DON displayed little loss during lyophilization in Fellman et al.'s (2008) tests, seemingly a perfect approach to DON preservation. However, the analytical error in this study (0.18 mg N/L) was almost equal to the concentration of DON (0.19 mg N/L) in the waters tested, which varied from 0.05 to 0.59 mg N/L. Such high analytic variability undermines the assumption that DON was less affected than DOC and TDP (Fellman et al., 2008). In another investigation, lyophilization followed by dialysis led to a lower recovery of DON than dialysis alone, indicating that freeze-drying can contribute significantly (33% ± 2%) to DOM loss (Vandenbruwane et al., 2007) (Table 1).

Several studies have questioned the effects of freeze-drying on the integrity of organic materials. The fluorescent and UV spectroscopic characteristics of organic matter were found to be remarkably altered if the samples had a DOC concentration greater than 5 mg C/L and/or if their SUVA is greater than 3.5 L/m mg-C (Spencer et al., 2007; Fellman et al., 2008; Hudson et al., 2009). The biodegradability of DOM, in terms of the lag phase and period needed to reach maximum respiration activity, was also modified after freezing (Pognani et al., 2012). This evidence shows that lyophilization is likely to alter DOM content and properties, and these potential side effects should be considered in concentration determination and avoided in sample characterization.

2.4.2. ROTARY EVAPORATION

RE, namely vacuum evaporation, uses a centrifugal force, heat, and vacuum to remove moisture and concentrate solutes. It differs from lyophilization in two aspects: first, water reduction via RE is from liquid to vapor, and second, a RE device is operated at higher temperature and dehydrates samples faster than freeze-drying.

The recovery of DON by RE has been evaluated but remains inconclusive (Table 1). Occasionally, the concentration process has been found to have an insignificant effect on DON recovery (Chang and Wang, 2013). Even when dialysis and RE were combined, the overall DOC loss was less than 5% for a surface water sample under the 10 mm Hg pressure, <40°C operating conditions (Feuerstein et al., 1997). This suggests that RE might result in little DOM loss. However, some other studies provided contradictory information. An experiment conducted by Vandenbruwane et al. (2007) indicated that RE process (operated at ~49°C) resulted in more DON loss than freeze-drying process (operated at −53°C and 442 mbar), which had an average 14% DOC loss (Fellman et al., 2008). Evaporation of volatile organics and adsorption onto the centrifuge glassware are the key causes of DOM loss (Vandenbruwane et al., 2007). When RE is operated at a temperature <30°C (Gjessing et al., 1998) or a near-ambient temperature (20°C) (Gondar et al., 2008), it exhibits higher DOC recoveries than RO (Gjessing et al., 1998). Similarly, DON recovery under 30°C operation was higher than recoveries under 40°C or 50°C (Chang and Wang, 2013). Thus, temperature control may be a way to optimize organic matter recovery. Further efforts are needed to better understand the factors affecting DOM changes and accordingly optimize the performance of the RE process (Figure 3).

3. CONCLUSIONS AND RECOMMENDATIONS

Along with the recognition of the importance of DON in the environment, many efforts have been made to establish a reliable DON analytical method.

Various pretreatment methods for DIN and DON separation and/or concentration have been evaluated to enable more accurate and precise DON analysis. Although progress has been made, challenges still exist. This article reviewed 10 pretreatment methods with regard to their DIN removal, DON retention, and DON property preservation capacities (Table 1). Based on these discussions, several key points and suggestions are summarized below (Figure 1).

Dialysis probably has the smallest membrane MWCO in comparison with other membranes, and many laboratories have endorsed it for reliable DON detection. Its use, nonetheless, is time-consuming which is a significant issue. Among pressure-driven processes, DON analysis using NF has been tested only once; most (69–92%) DIN species were removed as well as some DOC (18–22%). ED and RO have not been investigated for DON analysis, but some evidence indicates that they have smaller MWCO and better performance than NF for DOM recovery. Therefore, they may merit evaluation as alternative methods in the future. In the authors' opinion, NF and RO are particularly suitable for samples with extremely low levels of DON (e.g., μg N/L in marine water) owing to their concurrent concentration and separation abilities; in comparison, dialysis and ED may be more useful for samples with very low DON/TDN ratios (e.g., <0.1 in nonnitrified municipal wastewater) because of their robust separation potentials and minor influence on DON quantity and quality. The applicabilities of these membrane methods are summarized in Figure 1.

Adsorptive methods, including IX resin, SEC, and nonionic XAD resins, have been evaluated in a few case studies. Although the results are promising, studies also reported significant amounts of DOM adsorption and changes in DOM characteristics during these processes. Therefore, a systematic investigation of these processes is probably necessary. The challenge of this type of method is to select an appropriate adsorbent that has little influence on DON but a strong preference for DIN removal. Figure 2 presents the features of these adsorptive methods.

Catalytic reduction of nitrate using bimetallic catalysis was unsuccessful for real water samples in many studies. Both adsorption and reaction of DON with catalytic agents may occur, which lead to unacceptable DON losses. In addition, the presence of competing compounds such as sulfate and sulfur-containing organic matter may interfere with the reactions. Future research, if worthwhile, should aim to identify catalysts with stronger reaction selectivity.

Lyophilization and RE were previously presumed to preserve DOM; however, a few more recent studies indicate that they cause DOM loss and chemical property changes (Figure 3). Researchers intending to use these methods for DON detection and characterization need to be aware of their limits and check their applicability under specific conditions. Based on the existing literature, future studies may want to evaluate the feasibility of low-temperature evaporation for sample concentration and preservation.

In addition to studying the effects of pH, temperature, time, and other environmental conditions that need to be further verified, researchers may also employ certified reference samples, such as samples from the International Humic Substance Society (IHSS), in future studies so that a widespread comparison among laboratories is possible. In addition, although they are not discussed in detail here, certain advanced characterization methods, such as FEEM (fluorescence excitation-emission matrix), NMR (nuclear magnetic resonance), and FTIR (Fourier transform infrared spectroscopy), are probably necessary to keep track of the DOM integrity (Matilainen et al., 2011).

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REFERENCES

Abou-Shady, A., Peng, C. S., Almeria, J., and Xu, H. Z. (2012). Effect of pH on separation of Pb (II) and NO_3^- from aqueous solutions using electrodialysis. *Desalination*, 285, 46–53.

Aluwihare, L. I., and Meador, T. (2008). *Nitrogen in the marine environment* (2nd ed., pp. 95–140). San Diego, CA: Academic Press.

Ambonguilat, S., Gallard, H., Garron, A., Epron, F., and Croue, J. P. (2006). Evaluation of the catalytic reduction of nitrate for the determination of dissolved organic nitrogen in natural waters. *Water Research*, 40(4), 675–682.

APHA, AWWA, and WEF. (1998). *Standard methods for the examination of water and wastewater*. Washington, DC: American Public Health Association.

Aufdenkampe, A. K., Hedges, J. I., Richey, J. E., Krusche, A. V., and Llerena, C. A. (2001). Sorptive fractionation of dissolved organic nitrogen and amino acids onto fine sediments within the Amazon Basin. *Limnology and Oceanography*, 46(8), 1921–1935.

Bachmann, R., and Canfield, D., Jr. (1996). Use of an alternative method for monitoring total nitrogen concentrations in Florida lakes. *Hydrobiologia*, 323(1), 1–8.

Bhatnagar, A., and Sillanpää, M. (2011). A review of emerging adsorbents for nitrate removal from water. *Chemical Engineering Journal*, 168(2), 493–504.

Bronk, D. A., and Glibert, P. M. (1991). A N^{15} tracer method for the measurement of dissolved organic nitrogen release by phytoplankton. *Marine Ecology Progress Series*, 77(2–3), 171–182.

Bronk, D. A., Glibert, P. M., and Ward, B. B. (1994). Nitrogen uptake, dissolved organic nitrogen release, and new production. *Science*, 265, 1843–1846.

Bronk, D. A., Lomas, M. W., Glibert, P. M., Schukert, K. J., and Sanderson, M. P. (2000). Total dissolved nitrogen analysis: Comparisons between the persulfate, UV and high temperature oxidation methods. *Marine Chemistry*, 69(1–2), 163–178.

Bronk, D. A., See, J. H., Bradley, P., and Killberg, L. (2007). DON as a source of bioavailable nitrogen for phytoplankton. *Biogeosciences*, 4(3), 283–296.

Buffle, J. (1988). *Complexation reactions in aquatic systems: An analytical approach*. New York: Ellis Horwood.

Burdige, D. J., and Zheng, S. (1998). The biogeochemical cycling of dissolved organic nitrogen in estuarine sediments. *Linology and Ocenography*, 43(8), 1796–1813.

Canfield, D. E., Glazer, A. N., and Falkowski, P. G. (2010). The evolution and future of earth's nitrogen cycle. *Science*, 330(6001), 192–196.

Cape, J. N., Cornell, S. E., Jickells, T. D., and Nemitz, E. (2011). Organic nitrogen in the atmosphere—Where does it come from? A review of sources and methods. *Atmospheric Research*, 102(1–2), 30–48.

Chang, H., and Wang, G. (2013). Fractionation of nitrogen-enriched dissolved organic matter in water. *Separation and Purification Technology*, 117, 89–97.

Chen, B., Kim, Y., and Westerhoff, P. (2011). Occurrence and treatment of wastewater-derived organic nitrogen. *Water Research*, 45(15), 4641–4650.

Crum, R. H., Murphy, E. M., and Keller, C. K. (1996). A non-adsorptive method for the isolation and fractionation of natural dissolved organic carbon. *Water Research*, 30(5), 1304–1311.

Crumpton, W. G., Isenhart, T. M., and Mitchell, P. D. (1992). Nitrate and organic N analyses wtih second-derivative spectroscopy. *Limnology and Oceanography*, 37(4), 907–913.

De Schamphelaere, K. A. C., Unamuno, V. I. R., Tack, F. M. G., Vanderdeelen, J., and Janssen, C. R. (2005). Reverse osmosis sampling does not affect the protective effect of dissolved organic matter on copper and zinc toxicity to freshwater organisms. *Chemosphere*, 58(5), 653–658.

Donnermair, M. M., and Blatchley, E. R. (2003). Disinfection efficacy of organic chloramines. *Water Research*, 37(7), 1557–1570.

Dotson, A., and Westerhoff, P. (2009). Occurrence and removal of amino acids during drinking water treatment. *Journal of American Water Works Association*, 101(9), 101–115.

Egeberg, P. K., Eikenes, M., and Gjessing, E. T. (1999). Organic nitrogen distribution in NOM size classes. *Environment International*, 25(2/3), 225–236.

Fellman, J. B., D'Amore, D. V., and Hood, E. (2008). An evaluation of freezing as a preservation technique for analyzing dissolved organic C, N and P in surface water samples. *Science of The Total Environment*, 392(2–3), 305–312.

Feuerstein, T. P., Ostrom, P. H., and Ostrom, N. E. (1997). Isotopic biogeochemistry of dissolved organic nitrogen: A new technique and application. *Organic Geochemistry*, 27(7–8), 363–370.

Flowers, R. C., and Singer, P. C. (2013). Anion exchange resins as sources of nitrosamines and nitrosamine precursors. *Environmental Science & Technology*, 47, 7365–7372.

Giesy, J. P., and Briese, L. A. (1978). Particulate formation due to freezing humic waters. *Water Resources Research*, 14(3), 542–544.

Gjessing, E. T., Alberts, J. J., Bruchet, A., Egeberg, P. K., Lydersen, E., McGown, L. B., Mobed, J. J., Münster, U., Pempkowiak, J., Perdue, M., Ratnawerra, H., Rybacki, D., Takacs, M., and Abbt-Braun, G. (1998). Multi-method characterisation of natural organic matter isolated from water: Characterisation of reverse osmosis-isolates from water of two semi-identical dystrophic lakes basins in Norway. *Water Research*, 32(10), 3108–3124.

Gondar, D., Thacker, S. A., Tipping, E., and Baker, A. (2008). Functional variability of dissolved organic matter from the surface water of a productive lake. *Water Research*, 42(1–2), 81–90.

Graeber, D., Gcker, B., Zwirnmann, E., Kronvang, B., Weih, C., and Gelbrecht, J. (2012a). Dialysis is superior to anion exchange for removal of dissolved inorganic nitrogen from freshwater samples prior to dissolved organic nitrogen determination. *Environmental Chemistry*, 9(6), 529–536.

Graeber, D., Gelbrecht, J., Kronvang, B., Gücker, B., Pusch, M. T., and Zwirnmann, E. (2012b). Technical Note: Comparison between a direct and the standard, indirect method for dissolved organic nitrogen determination in freshwater environments with high dissolved inorganic nitrogen concentrations. *Biogeosciences*, 9(11), 4873–4884.

Guldberg, L. B., Finster, K., Jorgensen, N. O.G., Middelboe, M., and Lomstein, B. A. (2002). Utilization of marine sedimentary dissolved organic nitrogen by native anaerobic bacteria. *Limnology and Oceanography*, 47(6), 1712–1722.

Hedges, J. I., Bergamaschi, B. A., and Benner, R. (1993). Comparative analyses of DOC and DON in natural waters. *Marine Chemistry*, 41(1–3), 121–134.

Hopkinson, C., Cifuentes, L., Burdige, D., Fitzwater, S., Hansell, D., Henrichs, S., Kähler, P., Koike, I., Walsh, T., and Bergamaschi, B. (1993). DON subgroup report. *Marine Chemistry*, 41(1–3), 23–36.

Huber, S. A., Balz, A., Abert, M., and Pronk, W. (2011). Characterisation of aquatic humic and non-humic matter with size-exclusion chromatography—organic carbon detection—organic nitrogen detection (LC-OCD-OND). *Water Research*, 45(2), 879–885.

Hudson, N., Baker, A., Reynolds, D. M., Carliell-Marquet, C., and Ward, D. (2009). Changes in freshwater organic matter fluorescence intensity with freezing/thawing and dehydration/rehydration. *Journal of Geophysical Research: Biogeosciences*, 114(G4), G00F08.

Johnes, P. J., and Heathwaite, A. L. (1992). A procedure fo the simultaneous determination of total nitrogen and total phosphorus in freshwater samples using persulfate microwave digestion. *Water Research*, 26(10), 1281–1287.

Kemper, J. M., Westerhoff, P., Dotson, A., and Mitch, W. A. (2008). Nitrosamine, dimethylnitramine, and chloropicrin formation during strong base anion-exchange treatment. *Environmental Science and Technology*, 43(2), 466–472.

Kilduff, J. E., Mattaraj, S., Wigton, A., Kitis, M., and Karanfil, T. (2004). Effects of reverse osmosis isolation on reactivity of naturally occurring dissolved organic matter in physicochemical processes. *Water Research*, 38(4), 1026–1036.

Kim, D. H., Moon, S.-H., and Cho, J. (2003). Investigation of the adsorption and transport of natural organic matter (NOM) in ion-exchange membranes. *Desalination*, 151(1), 11–20.

Kitis, M., Kilduff, J. E., and Karanfil, T. (2001). Isolation of dissolved organic matter (DOM) from surface waters using reverse osmosis and its impact on the reactivity of dom to formation and speciation of disinfection by-products. *Water Research*, 35(9), 2225–2234.

Koprivnjak, J. F., Perdue, E. M., and Pfromm, P. H. (2006). Coupling reverse osmosis with electrodialysis to isolate natural organic matter from fresh waters. *Water Research*, 40(18), 3385–3392.

Kotlash, A. R., and Chessman, B. C. (1998). Effects of water sample preservation and storage on nitrogen and phosphorus determinations: Implications for the use of automated sampling equipment. *Water Research*, 32(12), 3731–3737.

Lara, R. J., Hubberten, U., Thomas, D. N., Baumann, M. E.M., and Kattner, G. (1997). Dissolved organic matter studies in enclosed systems: Application of hydrophobic fractionation for the assessment of organic nitrogen dynamics. *Journal of Marine Systems*, 13(1–4), 155–161.

Lee, H.-J., Kim, D. H., Cho, J., and Moon, S.-H. (2003a). Characterization of anion exchange membranes with natural organic matter (NOM) during electrodialysis. *Desalination*, 151(1), 43–52.

Lee, S., Cho, J., Shin, H., Son, B., and Chae, S. (2003b). Investigation of NOM size, structure and functionality (SSF): Impact on water treatment process with respect to disinfection by-products formation. *Journal of Water Supply: Research and Technology-AQUA*, 52(8), 555–564.

Lee, W. (2005). *Occurrence, molecular weight and treatability of dissolved organic nitrogen* (dissertation). Arizona State University, Phoenix.

Lee, W., and Westerhoff, P. (2004). Dissolved organic nitrogen measurement using dialysis pretreatment. *Environmental Science and Technology*, 39(3), 879–884.

Lee, W., Westerhoff, P., and Esparza-soto, M. (2006). Occurrence and removal of dissolved organic nitrogen in US water treatment plants. *Journal of American Water Works Association*, 98(10), 102–110.

Leenheer, J. A., Dotson, A., and Westerhoff, P. (2007). Dissolved organic nitrogen fractionation. *Annals of Environmental Science*, 1, 45–56.

Liou, Y. H., Lin, C. J., Hung, I. C., Chen, S. Y., and Lo, S. L. (2012). Selective reduction of NO_3^- to N_2 with bimetallic particles of Zn coupled with palladium, platinum, and copper. *Chemical Engineering Journal*, 181–182, 236–242.

Liu, H., Jeong, J., Gray, H., Smith, S., and Sedlak, D. L. (2011). Algal uptake of hydrophobic and hydrophilic dissolved organic nitrogen in effluent from biological nutrient removal municipal wastewater treatment systems. *Environmental Science and Technology*, 46(2), 713–721.

Matilainen, A., Gjessing, E. T., Lahtinen, T., Hed, L., Bhatnagar, A., and Sillanpää, M. (2011). An overview of the methods used in the characterisation of natural organic matter (NOM) in relation to drinking water treatment. *Chemosphere*, 83(11), 1431–1442.

Maurice, P. A., Pullin, M. J., Cabaniss, S. E., Zhou, Q., Namjesnik-Dejanovic, K., and Aiken, G. R. (2002). A comparison of surface water natural organic matter in raw filtered water samples, XAD, and reverse osmosis isolates. *Water Research*, 36(9), 2357–2371.

McCarthy, M. D., Hedges, J. I., and Benner, R. (1998). Major bacterial contribution to marine dissolved organic nitrogen. *Science*, 281(5374), 231–234.

Murphy, D. V., Macdonald, A. J., Stockdale, E. A., Goulding, K. W.T., Fortune, S., Gaunt, J. L., Poulton, P. R., Wakefield, J. A., Webster, C. P., and Wilmer, W. S. (2000). Soluble organic nitrogen in agricultural soils. *Biology and Fertility of Soils*, 30(5–6), 374–387.

Pehlivanoglu-Mantas, E., and Sedlak, D. L. (2006). Wastewater-derived dissolved organic nitrogen: Analytical methods, characterization, and effects—A review. *Critical Reviews in Environmental Science and Technology*, 36(3), 261–285.

Perdue, E. M., and Ritchie, J. D. (2003). Dissolved Organic Matter in Freshwaters. In: *Treatise on geochemistry*. D. H. Heinrich and K. T. Karl (Eds.), (pp. 273–318). Oxford: Pergamon.

Peuravuori, J., and Pihlaja, K. (1997). Isolation and characterization of natural organic matter from lake water: Comparison of isolation with solid adsorption and tangential membrane filtration. *Environment International*, 23(4), 441–451.

Pognani, M., Barrena, R., Font, X., and Sánchez, A. (2012). Effect of freezing on the conservation of the biological activity of organic solid wastes. *Bioresource Technology*, 104, 832–836.

Runge, S. W., Shelton, K. R., Melton, S. A., and Moran, W. M. (2005). Maintaining the ionic permeability of a cellulose ester membrane. *Journal of Biochemical and Biophysical Methods*, 64(3), 200–206.

Sattayatewa, C., Arnaldos, M., and Pagilla, K. (2011). Measurement of organic nitrogen and phosphorus fractions at very low concentrations in wastewater effluents. *Water Environment Research*, 83(8), 675–683.

Schulten, H. R., and Schnitzer, M. (1997). The chemistry of soil organic nitrogen: A review. *Biology and Fertility of Soils*, 26(1), 1–15.

Schwede-Thomas, S., Chin, Y.-P., Dria, K., Hatcher, P., Kaiser, E., and Sulzberger, B. (2005). Characterizing the properties of dissolved organic matter isolated by XAD and C-18 solid phase extraction and ultrafiltration. *Aquatic Sciences*, 67(1), 61–71.

Sharp, J. H., Rinker, K. R., Savidge, K. B., Abell, J., Benaim, J. Y., Bronk, D., Burdige, D. J., Cauwet, G., Chen, W., Doval, M. D., Hansell, D., Hopkinson, C., Kattner, G., Kaumeyer, N., McGlathery, K. J., Merriam, J., Morley, N., Nagel, K., Ogawa, H., Pollard, C., Pujo-Pay, M., Raimbault, P., Sambrotto, R., Seitzinger, S., Spyres, G., Tirendi, F., Walsh, T. W., and Wong, C. S. (2002). A preliminary methods comparison for measurement of dissolved organic nitrogen in seawater. *Marine Chemistry*, 78, 171–184.

Siddiqui, M., Amy, G., Ryan, J., and Odem, W. (2000). Membranes for the control of natural organic matter from surface waters. *Water Research*, 34(13), 3355–3370.

Solinger, S., Kalbitz, K., and Matzner, E. (2001). Controls on the dynamics of dissolved organic carbon and nitrogen in a Central European deciduous forest. *Biogeochemistry*, 55(3), 327–349.

Spencer, R. G.M., Bolton, L., and Baker, A. (2007). Freeze/thaw and pH effects on freshwater dissolved organic matter fluorescence and absorbance properties from a number of UK locations. *Water Research*, 41(13), 2941–2950.

Sun, L., Perdue, E. M., and McCarthy, J. F. (1995). Using reverse osmosis to obtain organic matter from surface and ground waters. *Water Research*, 29(6), 1471–1477.

van Kessel, C., Clough, T., and van Groenigen, J. W. (2009). Dissolved organic nitrogen: An overlooked pathway of nitrogen loss from agricultural systems? *Journal of Environmental Quality*, 38(2), 393–401.

Vandenbruwane, J., De Neve, S., Qualls, R. G., Salomez, J., and Hofman, G. (2007). Optimization of dissolved organic nitrogen (DON) measurements in aqueous samples with high inorganic nitrogen concentrations. *Science of the Total Environment*, 386(1–3), 103–113.

Wang, Y.-N., and Tang, C. Y. (2011). Protein fouling of nanofiltration, reverse osmosis, and ultrafiltration membranes—The role of hydrodynamic conditions, solution chemistry, and membrane properties. *Journal of Membrane Science*, 376(1–2), 275–282.

Westerhoff, P., Lee, W., Croué, J.-P., Gallard, H., and Amy, G. (2006). *Organic nitrogen in drinking water and reclaimed wastewater*. Colorado, USA: Awwa Research Foundation.

Westerhoff, P., and Mash, H. (2002). Dissolved organic nitrogen in drinking water supplies: A review. *Journal of Water Supply Research and Technology-AQUA*, 51(8), 415–448.

Worsfold, P. J., Monbet, P., Tappin, A. D., Fitzsimons, M. F., Stiles, D. A., and McKelvie, I. D. (2008). Characterisation and quantification of organic phosphorus and organic nitrogen components in aquatic systems: A review. *Analytica Chimica Acta*, 624(1), 37–58.

Xu, B., Li, D.-P., Li, W., Xia, S.-J., Lin, Y.-L., Hu, C.-Y., Zhang, C.-J., and Gao, N.-Y. (2010). Measurements of dissolved organic nitrogen (DON) in water samples with nanofiltration pretreatment. *Water Research*, 44(18), 5376–5384.

Yang, X., Shang, C., and Westerhoff, P. (2007). Factors affecting formation of haloacetonitriles, haloketones, chloropicrin and cyanogen halides during chloramination. *Water Research*, 41(6), 1193–1200.

Zhang, Y., Love, N., and Edwards, M. (2009). Nitrification in drinking water systems. *Critical Reviews in Environmental Science and Technology*, 39(3), 153–208.