

Soluble Organic Nitrogen in Biological Nutrient Removal



MARCH 2019

The 2018 *Dissolved Organic Nitrogen (DON) in Biological Nutrient Removal Wastewater Treatment Processes Compendium* was written to identify knowledge gaps to be addressed by the Nutrient Removal Challenge. That document contains state-of-the-art knowledge to achieve reliable, cost-effective nutrient removal. The 2018 compendium included a number of questions and challenges to reduce nutrients in advanced treated wastewater. This 2019 compendium revision contains a summary of the findings presented in reports and documents generated by researchers and contributors.

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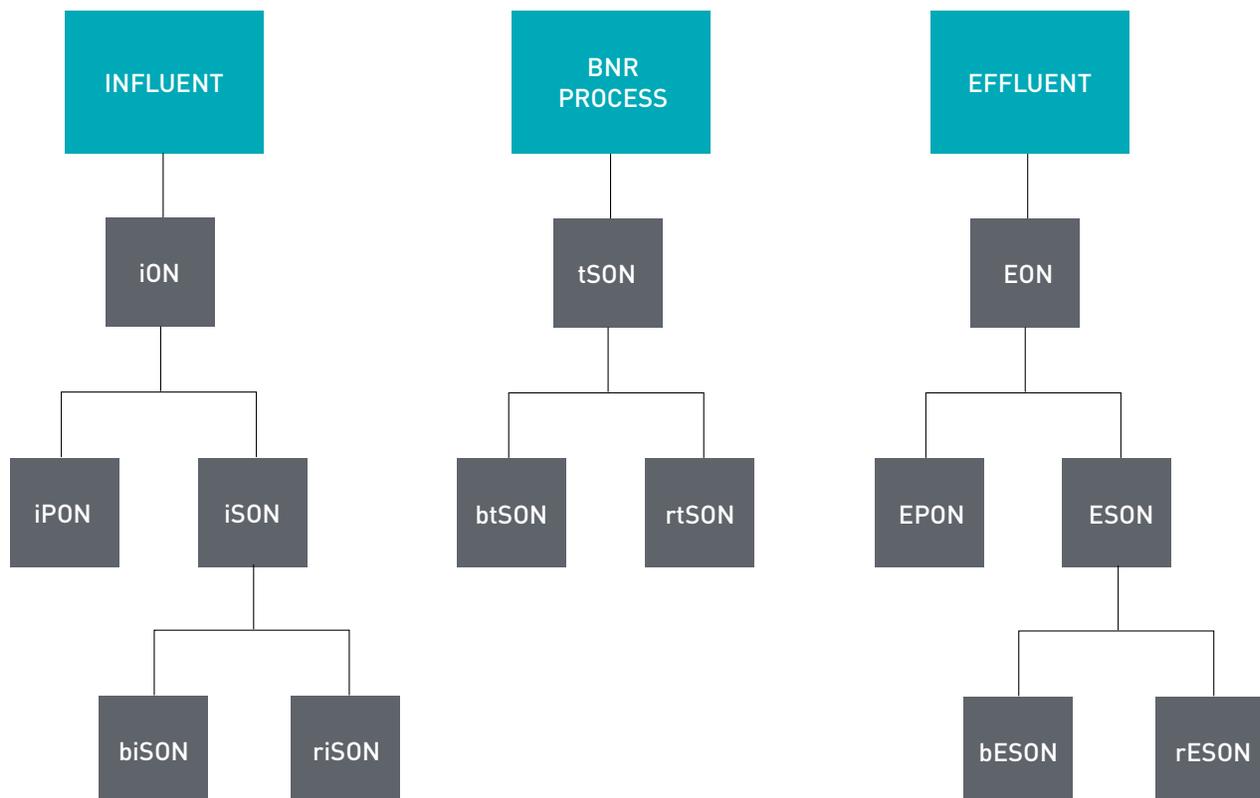
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DEFINITIONS AND ACRONYMS

The organic nitrogen constituents of interest are shown below in Figure 1. The influent organic nitrogen (iON) equals the sum of the influent particulate organic nitrogen (iPON) and influent SON (iSON). The influent SON consists of biodegradable (biSON) and non-biodegradable or recalcitrant (riSON). The main organic nitrogen component of interest in the BNR treatment process is the SON, because most of the iPON will either be captured in solids removal processes or converted to SON. The SON in the BNR process is referred to as treatment process SON (tSON) and it consists of a biodegradable component (btSON) and a non-biodegradable component (rtSON). The organic nitrogen in the BNR process effluent is referred to as EON and this consists of particulate (EPON) and ESON. The soluble portion is defined by the effluent filtration pore size, with 0.45 μm commonly. The organic nitrogen in the filtrate is defined as soluble but it may also contain some colloidal organic nitrogen. Of interest for the ESON is what portion is available for algae growth (i.e., bioavailable—denoted bESON) and what portion is not available or recalcitrant (rESON). The difference between btSON and bESON is that the biodegradable SON in the BNR process is related only to bacteria activity and the effluent bESON is bioavailable effluent SON that involves activities of both bacteria and algae in surface waters. These acronyms are summarized below along with others used in this compendium.

Figure 1 - Organic Nitrogen Components of Interest in the BNR Process



AOP	Advanced Oxidation Process
biSON	Biodegradable Influent Soluble Organic Nitrogen
BNR	Biological Nutrient Removal – Includes biological process designs for nitrogen and phosphorus removal
bSON	Bioavailable SON – Soluble organic nitrogen that can be biodegraded in the biological treatment process or can be used in surface waters due to bacteria activity and algae uptake of nitrogen
bESON	Bioavailable SON to Algae and Bacteria in Surface Waters
btSON	Biodegradable Soluble Organic Nitrogen in BNR Treatment System
DO	Dissolved Oxygen
EON	Effluent Organic Nitrogen – Sum of SON and PON in wastewater treatment plant effluent
EPON	Effluent Particulate Organic Nitrogen
ESON	Effluent Soluble Organic Nitrogen
FeCl ₃	Ferric Chloride
GAC	Granular Activated Carbon
H ₂ O ₂	Hydrogen Peroxide
iON	Influent Organic Nitrogen
iPON	Influent Particulate Organic Nitrogen
iSON	Influent Soluble Organic Nitrogen
LOT	Limits of Technology
NH ₃ -N	Total Ammonia-nitrogen – Includes free ammonia (NH ₃) and ionized ammonium (NH ₄ ⁺)
NO ₂ -N	Nitrite-nitrogen
NO ₃ -N	Nitrate-nitrogen
NO _x -N	Nitrite-nitrogen Plus Nitrate-nitrogen
ON	Organic Nitrogen – Nitrogen contained in organic compounds, i.e., amino acids, peptides, and protein, and can be in soluble form or contained in particulate material
PAC	Powdered Activated Carbon
PON	Particulate Organic Nitrogen – Organic nitrogen contained in wastewater solids or biomass
riSON	Non-biodegradable Influent Soluble Organic Nitrogen
RO	Reverse Osmosis
rSON	Recalcitrant SON – Soluble organic nitrogen that is resistant to biological transformation and uptake by algae in surface waters
rESON	Recalcitrant Effluent SON
rtSON	Non-biodegradable Soluble Organic Nitrogen in BNR Treatment System

SCAA	Soluble Combined Amino Acids
SFAA	Soluble Free Amino Acids
SON	Soluble Organic Nitrogen – Organic nitrogen measured in the filtrate of a sample (influent, mixed liquor, or effluent) following filtration
SRT	Solids Retention Time – Average time in days that solids are in the activated sludge system. It can be based on aerobic volume only or total volume.
STAC	Scientific and Technical Advisory Committee
TIN	Total Inorganic Nitrogen – Sum of $\text{NO}_2\text{-N}$, $\text{NO}_3\text{-N}$, and $\text{NH}_3\text{-N}$
TKN	Total Kjeldhal Nitrogen – Measures sum of organic nitrogen and $\text{NH}_3\text{-N}$
TN	Total Nitrogen – Sum of inorganic and organic nitrogen as N
TOC	Total Organic Carbon
tSON	Soluble Organic Nitrogen in BNR Treatment System
UV	Ultraviolet
WRRF	Water Resources Recovery Facility
WWTP	Wastewater Treatment Plant

BACKGROUND

Many municipal water resources recovery facilities are being challenged to remove nitrogen and phosphorus to much lower effluent concentrations to help minimize eutrophication in surface waters. For nitrogen, point source discharge permits are typically based on limiting the effluent total nitrogen (TN) concentration, which includes organic and inorganic forms of nitrogen. Nitrogen in the influent to a water resources recovery facility (WRRF) consists of ammonia (NH₃-N), particulate organic nitrogen (PON), and soluble organic nitrogen (SON). Biological transformations in biological nutrient removal (BNR) systems result in an effluent TN consisting of soluble and particulate organic nitrogen and inorganic nitrogen components. The inorganic nitrogen components are ammonia (NH₃-N), nitrate (NO₃-N), and nitrite (NO₂-N). BNR processes are specifically designed to oxidize NH₃-N to NO₃-N and/or NO₂-N and to biologically reduce these compounds to nitrogen gas by biological denitrification. Complex hydrolysis and deamination processes convert organic nitrogen to NH₃-N. To meet more stringent nitrogen removal requirements, BNR processes are pushed to their limits of technology (LOT) to biologically transform NH₃-N, NO₃-N, and NO₂-N. BNR LOT processes are aimed at meeting effluent TN concentrations well below the more traditional goal of 10 mg/L. In many cases, an effluent TN concentration of 3.0 mg/L is considered to represent the LOT for biological nitrogen removal. At lower effluent TN concentrations, the effluent organic nitrogen (EON) concentration may account for 30 to 60 percent of the effluent TN concentration, which has resulted in increased interest about what it is, how it can be minimized in a BNR facility effluent, and what its impact is on eutrophication. Effluent suspended solids are minimal in facilities aiming for minimal effluent TN concentrations through their use of effluent filtration or membrane separation; thus, the effluent soluble organic nitrogen (ESON) is of primary focus.

The purpose of this compendium is to summarize key current information on influent, in-plant, and effluent SON characteristics, including the impact of ESON on effluent TN goals, the composition of ESON, how SON is removed or produced in BNR processes, what fraction of ESON is accessible by bacteria, what fraction of ESON is available for algae growth, and the significance of ESON to eutrophication in surface waters. The information is presented in a format to answer key questions about the fate of SON and ESON. This compendium began with a presentation of the information provided here to a collaborative team in a workshop by the Scientific and Technical Advisory Committee to the Chesapeake Bay Program and the Water Environment Research Foundation's (now WRF) Nutrient Removal Challenge—Establishing a Research Agenda for Assessing the Bioavailability of Wastewater-Derived Organic Nitrogen in Treatment Systems and Receiving Waters. Contributors to this compendium are listed in the acknowledgments. This compendium is updated from additional information provided by studies under the Nutrient Removal Challenge and is the last compendium upgrade related to work by the Nutrient Removal Challenge.

●●●● EFFLUENT NITROGEN COMPONENTS IN BNR PROCESSES

What Nitrogen Components Make Up the Effluent TN Concentration from a Biological Nutrient Removal Water Resources Recovery Facility?

Table 1 shows the effluent nitrogen constituents that contribute to the effluent TN concentration from a BNR treatment process and the BNR process mechanism and factors that affect the respective effluent concentration. Note that key process design parameters that affect the ability to achieve minimal effluent TN concentrations (LOT performance) from BNR systems are longer solids retention times (SRTs), carbon additions for NO₃-N and NO₂-N removal, and enhanced effluent solids removal by membrane separation or filtration. Other factors may be the impact of variable loadings due to seasonal or wet weather conditions and the impact of in-plant recycle streams such as nitrogen-rich centrate return.

Table 1 - BNR Effluent Nitrogen Constituents and Process Removal Mechanisms		
Nitrogen Constituent	Process Removal Mechanisms	Known Factors Affecting Ability to Reach Minimum Concentrations
NH ₃ -N	Nitrification	Temperature, pH, Dissolved Oxygen, SRT
NO ₂ -N	Oxidation to NH ₃ -N Denitrification	Temperature, pH, Dissolved Oxygen, SRT Temperature, MLVSS Concentration, Carbon Source, Anoxic Detention Time
NO ₃ -N	Denitrification	Temperature, MLVSS Concentration, Carbon Source, Anoxic Detention Time
ESON	Hydrolysis and Ammonification	Temperature, SRT
EPON	Clarification, Filtration, or Membrane Separation	Liquid-solids Separation Process Design

What Filter Pore Size Is Used to Define ESON, iSON, and tSON?

The SON concentration measured for influent, treatment process or effluent samples will depend on the filter pore size used to separate particulate and colloidal solids from a sample. The common filter size for “soluble constituents” is 0.45 µm and has been used to define ESON in many studies. In bioassays aimed at determining the biodegradable SON by bacteria in wastewater treatment processes (btSON) (Khan 2007) used a 0.20 µm filter size to separate SON as did Pehlivanoglu and Sedlak (2004) in early work to evaluate the uptake of SON to bacteria and algae (bSON). A 0.45 µm filter size was used for effluent bSON in the Nutrient Removal Challenge reports by Sedlak et al. (2013) and Li et al. (2015). The ESON after passing 0.45 µm filters (and possibly 0.20 µm filters) may not be truly soluble and may contain colloidal organic nitrogen. The only way to separate this from the truly soluble fraction is with ultrafiltration; to date, this has not been done.

The following data presented by Pagilla (2007) shows results on the effect of filtration pore size on the organic nitrogen concentration for effluents from a number of wastewater treatment plants. The last four plants in Table 2 are BNR systems in Poland and the contribution in effluent SON from colloidal particles between 0.10 μm and 0.45 μm filtration ranged from 0.6 to 0.9 mg/L. However, the ESON was similar for the first three U.S. plants for samples with 0.10 μm and 0.45 μm filtration. The composition of SON substance and its bioavailability may be related to whether it is the colloidal or truly soluble form.

Table 2 - ESON Measurements (mg/L) in WRRFs as a Function of Filter Pore Size			
Water Resource Recovery Facility	Filter Pore Size		
	1.2 μm	0.45 μm	0.10 μm
Stickney	2.9	1.7	1.6
Hinsdale	4.2	3.6	3.6
Elmhurst	2.1	2.0	2.0
Gdynia	3.4	2.4	1.5
Gdansk	1.9	1.3	0.4
Elblag	5.0	2.7	2.0
Slupsk	1.6	1.6	1.0

Source: Pagilla 2007

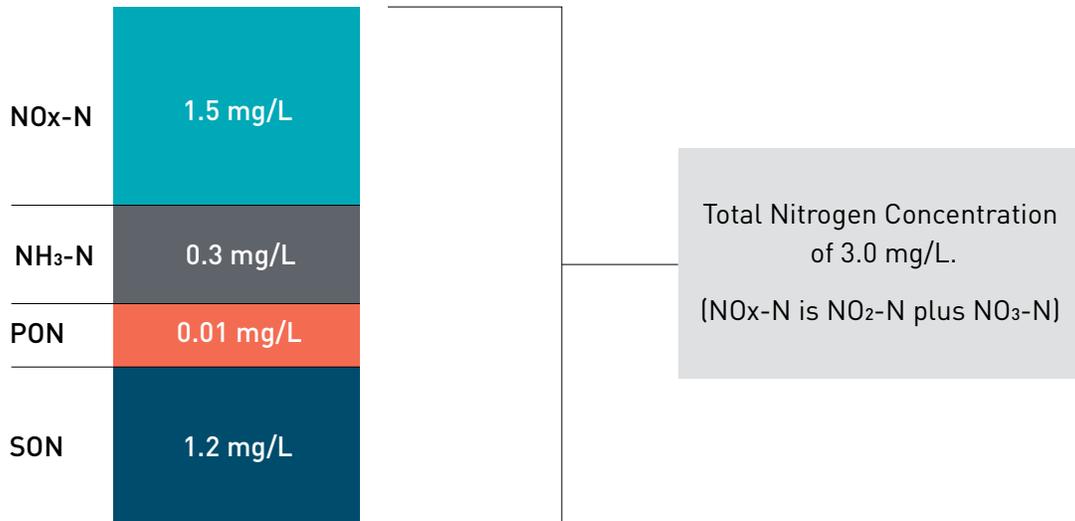
What Effluent TN Concentration Is Possible from a BNR LOT Process Designed and Operated for Maximum Nitrogen Removal? What Fraction of That Is ESON?

The Nutrient Removal Challenge final report (Neethling et al. 2018) addressed a number of factors that affect the possible effluent TN concentration from well designed and well operated BNR facilities aimed at minimizing effluent TN. From program studies on reported long-term plant performance variability (Bott and Parker 2011) and impacts of SON, the report shows that possible reliable effluent TN concentrations for such systems may range from 3.0 to 6.0 mg/L. Only a few facilities have been able to achieve monthly average effluent TN concentrations below 2.5 mg/L. No single minimal TN concentration value can be projected for all facilities as the effluent value is affected by influent flow and strength variations, equipment malfunctions, recycle streams, process design, and plant operations.

Figure 2 illustrates possible effluent nitrogen species concentrations from a BNR system able to achieve an effluent TN concentration of 3.0 mg/L. In this example, feasible effluent species concentration of 1.5, 0.3, and 1.2 mg/L are assumed for effluent $\text{NO}_x\text{-N}$ ($\text{NO}_2\text{-N}$ plus $\text{NO}_3\text{-N}$), $\text{NH}_3\text{-N}$, and SON, respectively. The ESON concentration accounted for 40 percent of the effluent TN concentration and thus is very significant for systems needing to meet minimum effluent

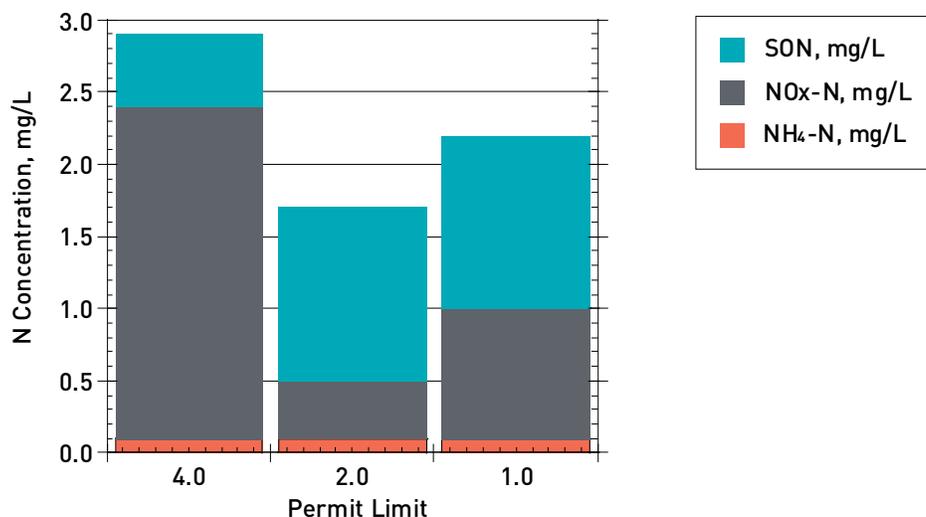
TN concentrations. For applications with an effluent TN concentration goal of less than 10 mg/L (typical value for water reuse applications), the ESON concentration is not as great of a concern.

Figure 2 - Possible Effluent Concentrations of Nitrogen Species in a BNR Effluent



Effluent nitrogen concentrations obtained from two Nutrient Removal Challenge reports (Bott and Parker 2011, Pellegrin et al. 2015) are presented for three facilities in Figure 3 to illustrate the importance of SON on the effluent TN concentration. The effluent TN concentration was higher for the system with the higher permit limit as this affected the operating conditions. Though one of the systems had an effluent permit of 1.0 mg/L, it was operating under an interim permit of 4.0 mg/L. The effluent SON for these facilities accounted for 17 percent, 70 percent, and 54 percent, respectively, of the effluent TN concentration. The effluent SON concentrations ranged from 0.5 to 1.2 mg/L for these facilities.

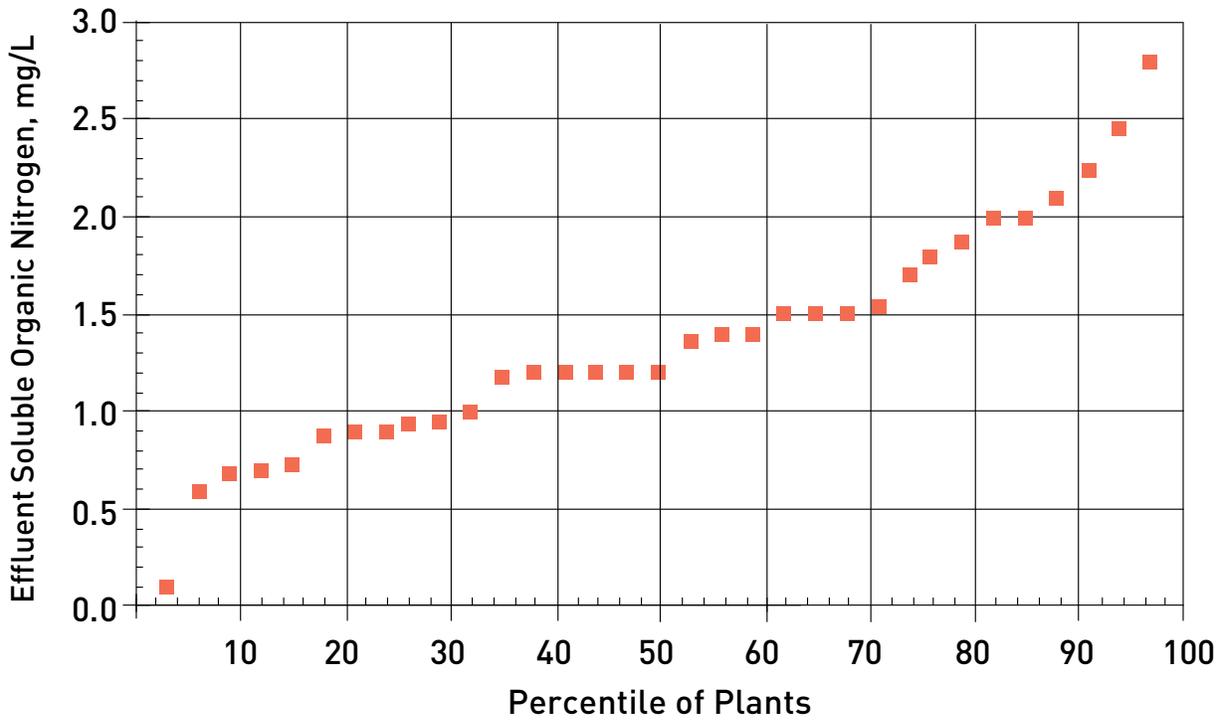
Figure 3 - Examples of Effluent Nitrogen Concentrations from WRRF Biological Nutrient Removal Systems Aimed at Meeting Different Effluent Permit Levels



What Are Some Observed ESON Concentrations in BNR Processes?

It appears that the effluent SON concentration is site specific. A ranking of effluent SON concentrations in Figure 4 from data reported assembled by Jimenez et al. (2007a) and tabulated in Table 3 shows effluent SON concentration ranged from 0.6 to 2.8 mg/L. The 50 percentile SON concentration for these data is 1.2 mg/L.

Figure 4 - Effluent SON Concentrations from Survey of Biological Nitrogen Removal Facilities



Source: Jimenez et al. 2007a

Table 3 - Summary of Effluent Soluble Organic Nitrogen Values Reported

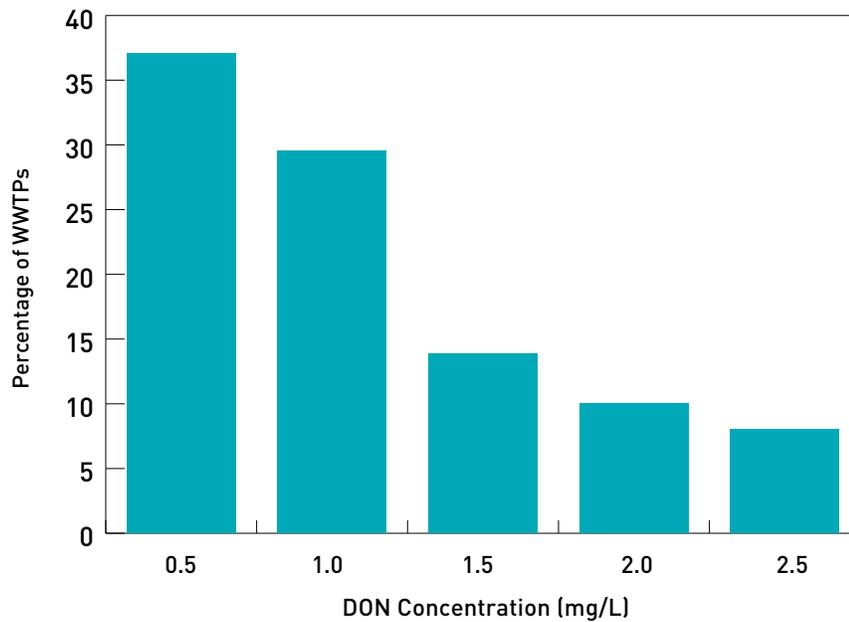
Plant Location	ESON (mg/L)	Percentile	Reference
Gorsonsville, VA	2.80	97	Pagilla 2007
Daytona Beach, FL, Bethune	2.46	94	Jimenez et al. 2007a
Back River WWTP	2.24	91	Parkin and McCarty 1981
New Smyrna, FL	2.10	88	Jimenez et al. 2007a
Daytona Beach, FL	2.00	85	Jimenez et al. 2007a
City of Bradenton, FL	2.00	82	Jimenez et al. 2007a
JEA Black Fords, FL	1.88	79	Jimenez et al. 2007a
City of Palmetto, FL	1.80	76	Jimenez et al. 2007a
Stamford, CT	1.70	74	Sharp and Brown 2007
Orange County, FL, Eastern	1.55	71	Jimenez et al. 2007a
Fort Meyers, FL, Central	1.50	68	Jimenez et al. 2007a
TMWRF, NV	1.50	65	Pagilla 2007
Palo Alto, CA (2)	1.50	62	Randtke and Mccarty 1977
Homestead, FL	1.40	59	Jimenez et al. 2007a
Lynn Haven, FL	1.40	56	Jimenez et al. 2007a
Bayou Marcus, FL	1.37	53	Jimenez et al. 2007a
City of Tarpon Springs, FL	1.20	50	Jimenez et al. 2007a
City of Clearwater, FL	1.20	47	Jimenez et al. 2007a
City of Largo, FL	1.20	44	Jimenez et al. 2007a
Chesapeake Beach, MD	1.20	41	Pagilla 2007
Blue Plains, D.C.	1.20	38	Pagilla 2007
City of Dunedin, FL	1.18	35	Jimenez et al. 2007a
Truckee Meadows, NV	1.00	32	Sedlak and Pehlivanoglu 2007
Titusville, FL	0.95	29	Jimenez et al. 2007a
Fort Meyers, FL, South	0.94	26	Jimenez et al. 2007a
Piscatway, MD	0.90	24	Pagilla 2007
Palo Alto, CA	0.90	21	Randtke and McCarty 1977
Orlando, FL	0.88	18	Jimenez et al. 2007a
Tampa, FL	0.73	15	Jimenez et al. 2007b
Alexandria, VA	0.70	12	O'Shaughnessy et al. 2006
Boone WWTP, VA	0.69	9	Wikramanayake et al. 2007
Fort Meyers, FL	0.60	6	Jimenez et al. 2007a
Upper Potomac R., MD	0.10	3	Pagilla 2007

* SON in Jimenez et al. 2007a reference estimated from effluent TN and TIN concentrations

Source: Jimenez et al. 2007a

A similar range of effluent SON concentrations was found by Pagilla (2007) and is shown in Figure 5. There is a wide range of observed ESON concentrations observed from BNR processes; it appears that in some cases the ESON can be at a high enough concentration to make it impossible to meet an effluent TN concentration goal of 3.0 mg/L.

Figure 5 - Summary of Effluent Soluble Organic Nitrogen Concentration (0.45 µm Filtration)



Source: Pagilla 2007

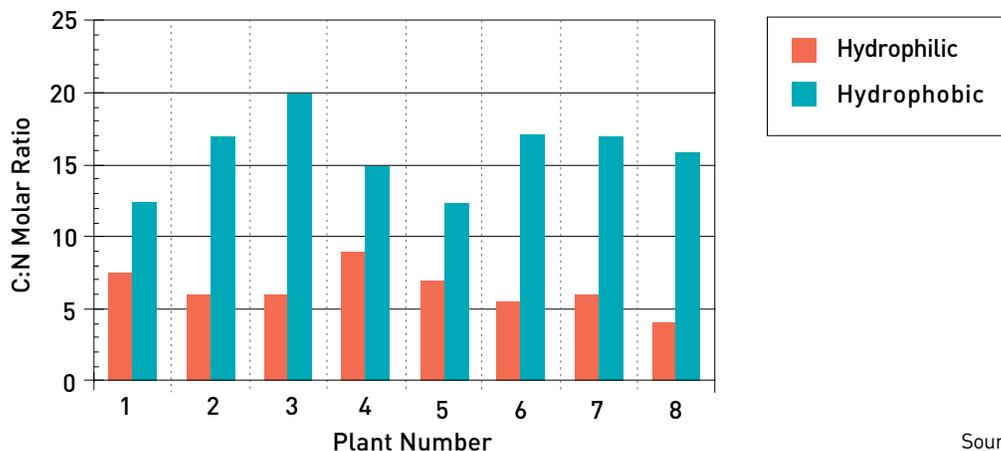
●●●● ESON CHARACTERISTICS

What Is the Composition of ESON?

Sedlak and Pehlivanoglu (2007) evaluated the molecular weight distribution of ESON and hypothesized that the high molecular fraction (molecular weight greater than 1 kilodalton) was not biologically available. The composition of this fraction has not been determined but is expected to be made up of larger molecular weight humic substances. Of the lower molecular weight compounds that may be bioavailable, only about one third has been identified as free and combined amino acids and ethylenediaminetetraacetic acid (EDTA). Other N-containing compounds in BNR effluents may include N-containing pesticides, pharmaceuticals, and other trace organics.

Evaluation of ESON in the Nutrient Removal Challenge study by Sedlak et al. (2013) found that the ESON could be classified as either hydrophilic or hydrophobic (Figure 6) and that the hydrophobic portion was likely made up of humic substances based on high C:N molar ratios of greater than 10.0. A Nutrient Removal Challenge study by Brett and Li (2015) on BNR system effluent also showed the presence of a humic-like substance for recalcitrant soluble organic phosphorus.

Figure 6 - Carbon: Nitrogen Ratios for Hydrophilic and Hydrophobic Species of Effluent SON from Biological Nitrogen Removal Facilities



Source: Sedlak et al. 2013

What Are Possible Sources of SON in BNR Facility Influent or in the Treatment Process?

SON originates in domestic wastewater as urea (60 to 80 percent of domestic influent TKN [total Kjeldhal nitrogen]), amino acids, proteins, aliphatic N compounds and synthetic compounds, such as EDTA. SON may also be produced and released in the wastewater treatment biological processes, including sludge digestion, due to cell metabolism processes that excrete biomolecules, cell decay, and cell lysis. Humic organic substances may be present in some drinking water supplies to eventually contribute to the wastewater SON. Little is known on industrial wastewater compounds that may contribute to SON in combined municipal-industrial wastewater treatment. Thus, ESON may consist of influent recalcitrant SON, SON produced by microbial activity in the BNR process, and biodegradable SON that remains in the effluent.

●●●● FATE OF SON IN BIOLOGICAL WASTEWATER TREATMENT

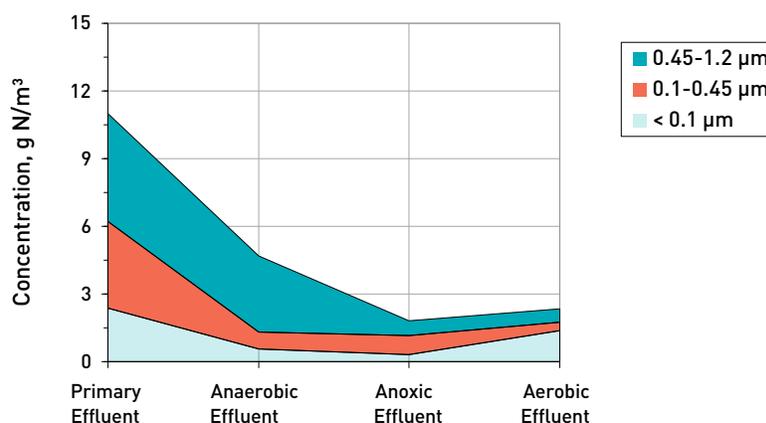
What is the Fate of ESON in Activated Sludge Treatment and BNR Treatment Processes?

In early work by Parkin and McCarty (1981), the composition and fate of SON at the Palo Alto, California wastewater treatment plant was studied. The average ESON concentration was 1.5 mg/L. They claimed that 52 percent of it was recalcitrant from influent wastewater sources, 20 percent was produced from biomass endogenous decay in the activated sludge process, 15 percent was in equilibrium between that sorbed to biomass and the liquid, and about 13 percent could be further degraded. However, they noted that increasing the activated sludge SRT could further degrade influent SON but SON could also be added via biomass endogenous respiration. They claimed that the optimal operating point that could lead to a minimal ESON concentration as a result of influent SON biodegradation and microbial SON release was at an SRT of 6 to 10 days. A number of important concepts regarding the fate of SON in wastewater treatment were revealed in this work: (1) some portion of the influent SON was not bioavailable, (2) increasing the system SRT could minimize the biodegradable SON concentration, and (3) increasing the SRT could increase the non-biodegradable SON concentration due to contributions from biomass endogenous decay.

In collaborative work with the Nutrient Removal Challenge, environmental engineering researchers at Gdansk University of Technology in Gdansk, Poland studied the fate of colloidal and soluble organic nitrogen in full scale facilities (Czerwionka et al. 2012). An example of the organic nitrogen results is shown in Figure 7 as a function of different filter pore sizes. Colloidal and SON nitrogen is removed in the anaerobic and anoxic zones, presumably by hydrolysis. SON is increased in the aerobic zone, presumably due to the release of microbial products from endogenous decay.

At present, there are no operating strategies to minimize the effluent SON concentration in advanced nutrient removal systems with biological treatment, chemical addition and effluent solids removal by filtration or membranes. Effluent SON concentration appears to be greatly affected by the influent wastewater characteristics, the biological process SRT, and internal return flows characteristics.

Figure 7 - Fate of Colloidal and Soluble Organic Nitrogen in Biological Nutrient Removal Facility



Source: Czerwionka et al. 2012

Return flow from dewatering anaerobic and aerobic digestion biosolids may be a source of high SON leading to higher effluent SON concentrations from BNR systems. During digestion, the destruction of waste activated sludge biomass can release intracellular proteins and possibly humic-like nitrogen compounds associated with the bacteria cell wall. The increase in SON during longer aeration time in the above reference to Czerwionka et al. (2012) also supports the potential for SON release in digestion operations. There was no opportunity to study the effect of digestion on effluent SON during the Nutrient Removal Challenge, but data from the plant performance studies (Bott and Parker 2011) for facilities that reported the lowest effluent TN concentrations (Table 4) show that BNR systems with primary treatment and anaerobic digestion had higher effluent SON than for plants without primary treatment and anaerobic digestion. This data is not conclusive, but raises issues with regard to the effect of anaerobic digestion with or without primary treatment on effluent SON in BNR facilities.

At present, there are no operating strategies to minimize the effluent SON concentration in advanced nutrient removal systems with biological treatment, chemical addition, and effluent solids removal by filtration or membranes. Effluent SON concentration appears to be greatly affected by the influent wastewater and internal return flows characteristics.

Table 4 - Comparison of Effluent SON Concentration and Sludge Processing Methods for Facilities with Very Low Effluent TN Concentration

Location	Design Flow, MGD	Process	Primary Treatment	Sludge Processing	Effluent SON mg/L	Effluent TN mg/L
Tahoe Truckee, CA	8.0	EBPR, N-BAF, DN-BAD, Ter. F	Yes	Anaerobic Digestion	1.7	2.5
Truckee Meadows, NV	46.5	NTF, DNUFB, Ter. F	Yes	Anaerobic Digestion	1.3	1.6
Broadrun, VA	11.0	Bardenpho-MBR, GAC	Yes	Anaerobic Digestion	1.2	2.2
Fiesta Village, FL	5.0	Oxidation Ditch, DNF	No	Anaerobic Digestion	0.9	1.0
Western Branch, MD	30.0	3 Sludge, C, Nitr. Denitri., Ter. F	No	Incineration	0.7	1.5

Source: Neethling et al. 2019

What Fraction of Influent SON Is Expected to Be Biodegradable SON?

Work reported by Khan (2007) suggested that 40 to 60 percent of influent SON is biodegradable. This is in the range of that given by Parkin and McCarty (1981) above. The relative effectiveness of different biological treatment process technologies on degrading influent or biomass-derived organic nitrogen has not been studied.

●●●● CONTROLLING AND MINIMIZING ESON FROM BNR FACILITIES

How Can the ESON Concentration of a Biological Nutrient Removal Facility Be Minimized?

Studies under the Nutrient Removal Challenge suggest that influent wastewater characteristics and possibly recycle streams have a larger role on ESON concentration than the advanced BNR process design and operating conditions. Studies did support the recommendation that excessively long SRT values beyond that needed for biological nitrogen and phosphorus removal should not be used because SON may be produced from microbial endogenous respiration. Waste sludge processing methods and recycle streams may also affect ESON concentration. These observations suggest that a tertiary SON removal step would be needed to further reduce ESON concentration.

What Process Technologies May Be Used for ESON Removal from a BNR Process Effluent and What Is the Effectiveness of These Processes?

Tertiary treatment processes that have been evaluated for SON removal are:

- Chemical Treatment
- Activated Carbon Adsorption
- Ion Exchange
- Advanced Oxidation
- Reverse Osmosis

Results from these studies are summarized below.

Chemical Treatment

Chemical treatment by coagulation-flocculation and liquid-solids separation is most often used in water treatment for removal of colloidal and particulate solids and in wastewater treatment for removal of phosphorus and effluent suspended solids. Metal salts in the form of ferric chloride (FeCl_3) and alum [$\text{Al}_2(\text{SO}_4)_3 \cdot 14\text{H}_2\text{O}$] are the most common coagulants in wastewater treatment. In chemical treatment, substances are removed from solution by sorption to the hydroxide floc produced and/or formation of metal hydroxide complexes. Because of the high hydrophilic fraction in effluent SON, high removal efficiency seems unlikely. This was confirmed by reported removal efficiencies for chemical treatment of secondary effluent SON summarized in Table 5. At high chemical dose of 200 to 300 mg/L, removal efficiencies ranged from 23 to 55 percent. Variations in performance between the test sites is likely due to differences in the effluent SON composition and/or chemical treatment procedures, including test pH and mixing methods.

Table 5 - Summary of SON Removal Performance for Chemical Treatment of Secondary Effluent

Reference	Initial SON mg/L	% SON Removal	Chemical	Dose mg/L	Test Samples
Randtke and McCarty 1979	1.4-3.8	23-31	Alum	200-300	Low SRT Activated Sludge
Bratby et al. 2008	0.4-1.6	30	FeCl ₃	300	Not Given
Chen et al. 2011	1.2-2.4	25	Alum	100	9 Full-scale U.S. Facilities
Czerwionka and Makinia 2014	1.0-1.3	10-20	FeCl ₃	100	3 Full-scale BNR, Poland
		23-41	FeCl ₃	200	
		47-55	FeCl ₃	300	

Activated Carbon Adsorption Treatment

Activated carbon adsorption has been used to remove soluble organic substances from wastewaters by passing the wastewater through upflow or downflow reactors containing granular activated carbon (GAC) of approximately 8 by 35 mesh. The carbon is periodically removed for thermal regeneration when the accumulation of the removed substances reaches a critical carbon loading capacity. Thus, the carbon bed contact time (hydraulic detention time) and the carbon loading capacity are important design and operating parameters.

The substrate loading capacity of the carbon (mg substrate sorbed/g carbon) varies with the carbon column bed depth, substrate concentration, and substrate chemistry. The substrate loading capacity is lower for lower final substrate concentrations. Substrate characteristics favorable for high activated carbon adsorption removal capacity are low solubility, less polarity or more hydrophobic, aromatic ring structure, and higher molecular weight.

Randtke and McCarty (1979) reported much better removal of SON using activated carbon than from chemical coagulation. The SON removal efficiency found in batch contacting experiments treating Palo Alto activated sludge treatment effluent with GAC was 71 percent. The batch contact time was very long (12 hours) and the carbon loading at this removal was 0.036 mg SON/g carbon, which is a low loading capacity compared to that observed for substrates that are known to be removed efficiently by activated carbon. For example, the loading capacity for phenol, at the same final substrate concentration of 0.38 mg/L achieved for SON removal in the Palo Alto samples, would be 1.12 mg phenol/g carbon or 31 times higher based on results reported by Karabacakoğlu et al. (2008).

Based on the observed carbon adsorption SON removal efficiency of 71 percent, Randtke and McCarty (1979) reasoned that the SON composition had more nonpolar compounds (hydrophobic) than polar compounds. However, Sedlak et al. (2013) found that the hydrophobic SON fraction of effluent from 10 BNR facilities ranged from 12 to 28 percent of the ESON concentration. Liu et al. (2012) also found that the hydrophobic portion of ESON from 12 BNR facilities was only 20 to 40 percent.

Results of other investigations on the ability of activated carbon to remove SON from activated sludge process effluent are summarized in Table 6. These were mixed, batch test carbon contacting experiments using secondary effluent samples. The carbon contact times and carbon doses were different in the different studies. The tests by Czerwionka and Makinia (2014) and Parkin and McCarty (1981) used GAC, while Chen et al. (2011) used powdered activated carbon (PAC). PAC typically has a higher loading capacity and faster removal rate than GAC due to the smaller size and greater exposed area to the bulk liquid. The much lower carbon capacity for Parkin and McCarty is due to using a much higher carbon dose than needed for removing the absorbable SON.

Czerwionka and Makinia (2014) were the only ones who investigated the effect of carbon dose and contact time. The results by Czerwionka and Makinia (2014) showed the expected behavior of activated carbon adsorption; removal efficiency increased with greater contact time and the carbon capacity was lower with a lower final SON concentration.

The carbon removal characteristics in the Chen et al. (2011) study was used to fit a Freundlich Isotherm, which was used to estimate the carbon capacity at different final SON concentrations in Table 6. The isotherm fits varied for the different secondary effluent sources. Equation 1 shows the isotherm fit for the nitrification-denitrification activated sludge system effluent fit for the laboratory activated sludge system is shown in Equation 2.

Equation 1:

$$q = 5.8C_e^{2.0}$$

Equation 2:

$$q = 6.5C_e^{1.5}$$

where:

q = carbon sorption capacity, mg SON sorbed/g activated carbon

C_e = equilibrium SON concentration, mg/L

The results by Chen et al. (2011) with PAC also show a difference in carbon capacity for different wastewaters. At an equilibrium concentration of 0.30 mg/L, the carbon capacity for the nitrification-

denitrification facility secondary effluent was 0.52 mg SON/g carbon compared to 1.07 mg SON/g carbon for the laboratory activated sludge effluent.

Table 6 - Summary of Results on Removal Efficiency and Carbon Loading from Batch Experiments to Assess the Ability to Remove Secondary Effluent SON by Activated Carbon Adsorption

Reference, Carbon Type	Initial SON mg/L	Carbon Dose mg/L	Contact Time Hours	% Removal	Final SON, mg/L	Carbon Capacity mg SON/g C
Czerwionka and Makinia, 2014 GAC	Gdynia Biological Nutrient Removal Facility Effluent					
	1.5	50	1.0	5	1.43	1.50
		200		12	1.32	0.90
		500		22	1.17	0.66
		50	3.0	18	1.23	5.40
		200		22	1.17	1.65
		500		35	0.98	1.05
		50	5.0	20	1.20	6.00
		200		30	1.05	2.25
		500		42	0.87	1.26
	Koscierzyna Biological Nutrient Removal Facility Effluent					
	1.0	50	1.0	20	0.80	4.00
		200		30	0.70	1.50
		500		40	0.60	0.80
		50	3.0	38	0.62	7.60
		200		58	0.42	2.90
		500		66	0.34	1.32
		50	5.0	44	0.56	8.80
		200		66	0.34	3.30
		500		70	0.30	1.40
Parkin and McCarty, 1981 GAC	1.14	50,000	12.0	65	0.40	0.01
Chen et al., 2011 PAC	Nitrification-Denitrification Activated Sludge Facility Effluent					
	2.44		4	80	0.50	1.45
				88	0.30	0.52
				92	0.20	0.23
	Laboratory Activated Sludge System Effluent					
	0.68		4	56	0.30	1.07
				70	0.21	0.60
			80	0.14	0.32	

The above studies with activated carbon removal of ESON show that the removal efficiencies may vary from 5 to 90 percent; thus, the expected performance is very site-specific. However, effluents with higher hydrophilic ESON fractions are expected to have lower treatment performance. Assessment of activated carbon treatment to remove ESON is best determined by bench and pilot-scale studies.

Ion Exchange Treatment

Ion exchange has been used for the removal of positive and negative charged dissolved substances and has been investigated for the removal of SON compounds that may be polar and hydrophilic. Parkin and McCarty (1981) showed a SON removal of only 23 percent using a cationic resin at neutral pH in batch test, but 33 to 35 percent removal at a pH of 2.0. They attributed the increased efficiency to more effective ion exchange removal of charged biologically produced amino acids, nucleic acids, and heterocyclic nitrogen compounds at low pH. Very little SON removal was also observed by Randtke and McCarty (1979) in batch tests with cation and anion exchange resins at neutral pH and by Czerwionka and Makinia (2014) with cation exchange at neutral pH. However, Randtke and McCarty (1979) also found increased SON removal with cation exchange at pH 2.0. They pointed out that ion exchange was effective for SON compounds not removed well by carbon adsorption and the potential for high SON removal by using a combination of activated carbon adsorption followed by treatment with cation exchange resin at a pH of 2.0. There have not been any studies on the use of this combined treatment scheme to reach a low final SON concentration. If it did work, a high carbon dose may still be expected.

Advanced Oxidation Treatment

An advanced oxidation process (AOP) is a chemical treatment process in which organic compounds are transformed due to oxidation through reactions with hydroxyl radicals. Common advanced oxidation processes are low-pressure ultraviolet (UV) treatment, hydrogen peroxide (H₂O₂) treatment, and a combination of UV and H₂O₂. AOP processes are able to convert nitrogen-containing organic compounds to simpler forms, which in some cases may be more biodegradable (Dwyer et al. 2008, Shah et al. 2013). The main interest in AOPs has been for the conversion of natural organic matter in water treatment or for conversion of specific micropollutants.

A short-term screening study by Gu and Tooker (2015) under the Nutrient Removal Challenge assessed the potential of advanced oxidation with post biotreatment to reduce ESON concentration from advanced BNR facilities. The effect of UV, H₂O₂, and UV/H₂O₂ treatment on SON conversion and increased biodegradability was studied with effluent samples from three facilities. The effluent SON concentrations from these samples ranged from 0.8 to 2.0 mg/L. The AOP dose ranges used were UV 1,000 to 2,000 mJ/cm² for UV and 5 to 10 mg/L for H₂O₂. The results of this study showed that a high UV and H₂O₂ dose is needed for AOP/biotreatment for ESON removal. UV or H₂O₂ alone did not significantly impact SON removal. The combination of UV/

H₂O₂ showed 15 to 64 percent conversion of major organic compounds related to ESON. Pilot plant or longer term bench-scale work is needed to assess the biodegradability of SON products.

Reverse Osmosis Treatment

In reverse osmosis (RO) treatment a high pressure has been used with semi-permeable membranes to cause water flow from a high dissolved solids concentration region to a low concentration region. The substances held back are termed rejected and the high concentration solution after water passage through the membrane is termed the reject stream. The membrane effluent is termed the permeate stream and the percent of applied water recovered in the permeate stream is termed the membrane recovery (Asano et al. 2006).

Initial interest in RO applications has been to obtain potable water from ocean or brackish water sources. More recently it has become an important process step in the production of potable water from wastewater treatment effluents (Qin et al. 2006). RO has also been identified as an alternative for the removal of SON because of its ability to remove over 95 percent of dissolved organic substances from treated wastewater. However, SON is only a small fraction of the total dissolved substances removed by RO and the reject stream flow may be as much as 20 percent of the feed stream flow. Important economic and operating issues associated with the use of RO are (1) management of the high total dissolved solids reject stream, (2) the high energy requirement, and (3) prevention and control of membrane fouling.

For most RO applications the most common parameters of followed have been total dissolved solids, organic carbon (TOC), conductivity, and specific ions such that there is limited data on SON removal. The fate of SON in RO applications is summarized in Table 7. The RO systems for the sites in Table 7 followed biological treatment and microfiltration or ultrafiltration membranes. The treatment needs for the facilities in Table 4 were mainly for indirect water reuse with the exception of the Escondido Hale Avenue Water Reclamation Facility. That study was done to assess the ability to meet a low effluent TN concentration for a portion of the Escondido The Hale Avenue Water Reclamation Facility flow.

Though RO also removes NH₄-N, NO₃-N, and NO₂-N ions, these are not removed as efficiently as large organic molecules. For the studies summarized in Table 7, the average RO removal efficiencies for NH₄-N and NO₃-N were 83 percent and 87 percent, respectively, compared to 92 percent for SON.

The results in Table 7 show the ability to consistently remove SON at high efficiency. Pilot-plant studies would be needed to evaluate the performance of ESON removal for a given site and to verify the performance and critical operating conditions, such as the system pressure and percent recovery claimed for specific RO supplier designs.

Table 7 - Summary of SON Concentrations in Reverse Osmosis Treatment Effluent in Full-scale and Pilot-plant Systems

Facility	Scale	Upstream Treatment	RO Influent mg SON/L	RO Permeate mg SON/L
GWRS	Full Scale	AS-TF-MF	2.03	0.10
LBWRP	Full Scale	BNR-Fil-MF	1.91	< 0.20
Scottsdale	Full Scale	BNR-MF	10.5	0.14
San Pasqual	Pilot	Pri-Hya-MF	0.67	< 0.20
		Pri-Hya-UF	0.67	< 0.20
Escondido	Pilot	AS-UF- 1	1.86	0.52
		AS-UF- 2	1.93	0.60
		AS-UF- 3	2.12	0.75
USBR	Pilot	MBR	0.76	0.10

Notes:

GWRS: Orange County Sanitation Groundwater Replenishment System

LBWRP: Leo J. Vender Lans Advanced Water Treatment Facility

Scottsdale: Scottsdale Water Campus

San Pasqual: San Pasqual Water Reclamation Facility

Escondido = Escondido Hale Avenue Water Reclamation Facility

USBR = US Bureau of Reclamation

AS=Activated Sludge (BOD)

TF = Trickling Filter

BNR = Biological Nutrient Removal

Pri-Hya = Primary treatment and Hyacinth Ponds

MF = Microfiltration

UF = Ultrafiltration

Source: Adapted from Merlo et al. 2012

Evaluation of RO for SON removal must consider the cost and operating requirements for handling the RO reject stream. Because of the high volume of the reject stream, processes that concentrate the stream further are of interest. These include multi-staged membrane separation, solar evaporation, and thermal evaporation methods. Ultimate disposal and permitting are of important concern and is site-specific (Asano et al. 2006).

●●●● FATE AND EFFECT OF EFFLUENT SON IN SURFACE WATERS

What Is the Importance of Nitrogen on Surface Water Quality?

Nitrogen can contribute to eutrophication, which can lead to low dissolved oxygen (DO) concentrations that hinder fish and shell fish production and survival. In fresh waters phosphorus is considered the most limiting inorganic nutrient but at elevated phosphorus concentrations higher algae growth can occur when more nitrogen is available. In saline waters, such as estuaries, phosphorus is plentiful so that the role of nitrogen is more important.

How Is the Nitrogen in ESON Used by Algae?

Hydrolysis and deamination of ESON can produce inorganic forms of nitrogen that are readily consumed by algae. Soluble free amino acids (SFAA) can be taken up directly by algae, but soluble combined amino acids (SCAA) must be hydrolyzed to monomers before uptake (Pehlivanoglu and Sedlak 2004). There is less known about the availability of nitrogen in larger molecular weight humic substances; however, in general, it is considered less available and has been termed inert or rSON. A study under the Nutrient Removal Challenge by Li et al. (2015) showed that the uptake of ESON by algae varied with a rapid uptake of some portion and an extremely slow uptake of the remaining SON, suggesting that a humic acid portion or more hydrophobic portion accounted for a recalcitrant SON in ESON from BNR facilities.

What Is Effluent Recalcitrant SON (rSON)?

The rSON is that portion of ESON that is considered not available for algal or bacterial growth over a time scale of days to weeks that may represent the time of travel through the water area of interest. This could involve only fresh water conditions or both fresh water and estuary saline water conditions; for example, the Chesapeake Bay and its tributaries. The structural characteristics of rSON are not known, but it is considered to be mainly in the unidentified high molecular weight humic fraction of effluent SON. However, for saline waters Mulholland et al. (2009) reports that humic compounds can be an available nitrogen source for algae growth. It is not known if the specific type of humic compounds and possibly other high molecular weight nitrogen compounds in BNR effluents are bioavailable in saline environments.

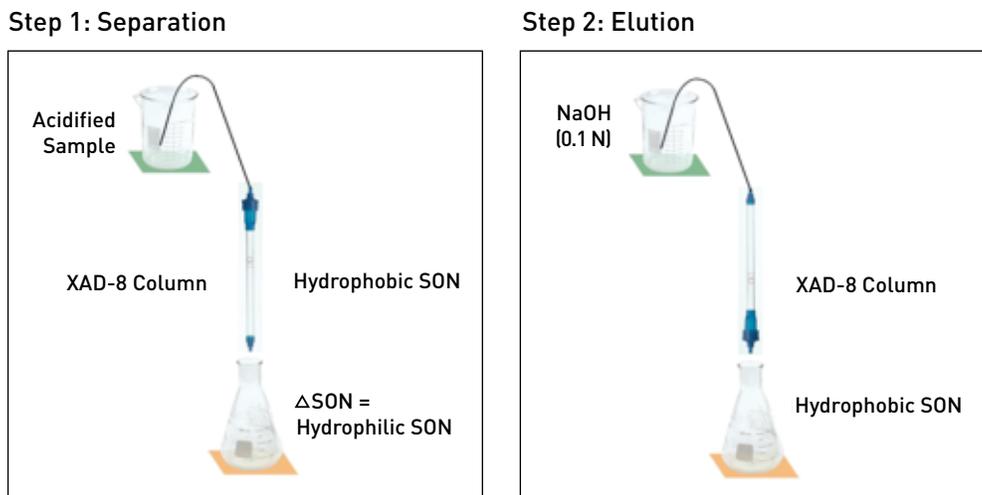
●●●● IMPACT OF rSON ON MEETING REGULATED EFFLUENT TN CONCENTRATIONS

What Fraction of Effluent SON from BNR Facilities May Be rSON?

In view of the wide range of ESON concentrations possible from BNR facilities, as shown in Table 3, it is not possible to generalize on the possible rSON fraction of ESON for all treatment plants. In early studies using a bioassay procedure in fresh water conditions with algae and bacteria, the fraction of ESON available for algae growth over a 14-day incubation period was 56 percent (Pehlivanoglu and Sedlak 2004) and 63 percent (Urgun-Demirtas et al. 2008) of the ESON for plants with low TN concentration effluents.

In a study under the Nutrient Removal Challenge, Sedlak et al. (2013) found that the fraction of rSON in ESON was related to the hydrophobic fraction. An ion exchange resin was used to separate the hydrophilic and hydrophobic portions of the ESON as shown in Figure 8. The bioavailability of hydrophilic SON and hydrophobic SON was then studied in a separate bioassay. The hydrophilic portion of the SON was found to be almost completely bioavailable in the bioassays, while the hydrophobic portion was only slightly bioavailable.

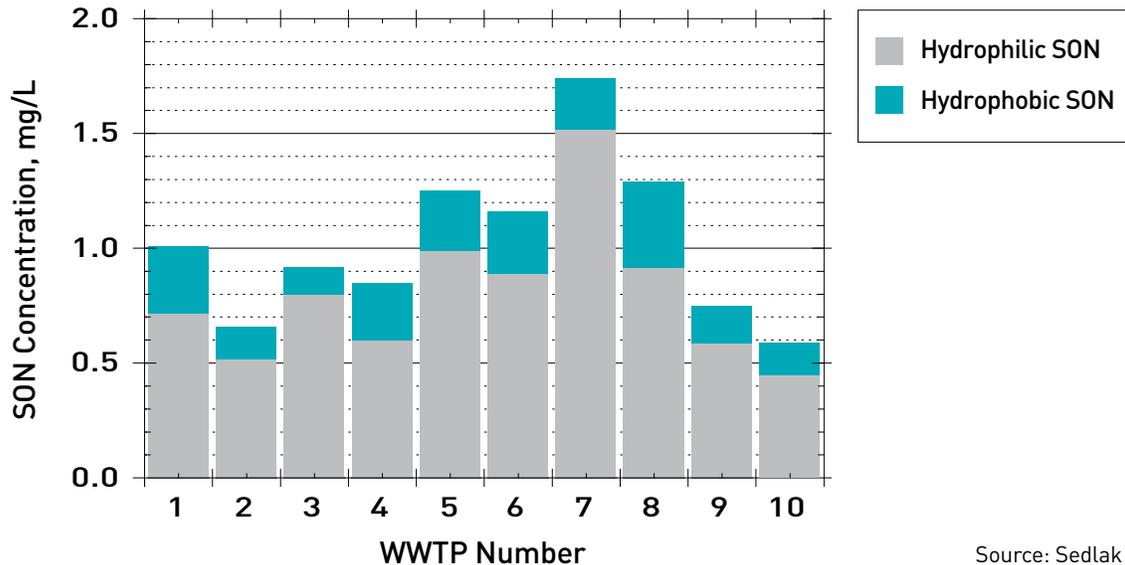
Figure 8 - An Ion Exchange Resin Method Separates Hydrophilic and Hydrophobic SON



Source: Sedlak et al. 2013

The ion exchange/bioassay procedure was applied with samples from ten BNR facilities, which were also analyzed for nitrogen species. All the facilities had nitrification and the effluent TN concentrations from 2.0 to 12.0 mg/L represented a wide range of nitrogen removal performance. The range of effluent NO_x-N and SON concentrations were from 0.10 to 11.2 mg/L, and 0.6 to 1.6 mg/L, respectively. Most of the effluent SON was hydrophilic as shown in Figure 9. The hydrophobic SON concentration for the ten facilities ranged from 0.15 to 0.37 mg/L, which was 12 to 28 percent of the ESON concentration. Thus, a large fraction of the ESON was bSON (72 to 88 percent).

Figure 9 - Hydrophobic and Hydrophilic SON Concentrations in Effluent SON at BNR Facilities



Source: Sedlak et al. 2013

How Significant Might Be the Effect of Effluent rSON on the Ability to Meet Stringent Effluent TN Concentration Permit Values?

For eutrophication impaired surface waters, a common regulated effluent TN concentration value is 3.0 mg/L. Based on the Nutrient Removal Challenge Program studies, effluent SON concentration for these plants could comprise 40 to 60 percent of the effluent TN and the rSON may be 8 to 16 percent of the ESON. Thus, the rSON may be only 5 to 7 percent of the effluent TN (0.15 to 0.21 mg/L, based on TN of 3.0 mg/L).

The results of the Nutrient Removal Challenge Program studies found that most of the ESON for low effluent TN discharges is bSON, which thus has a major role in meeting low effluent TN concentration. Much of the ESON is not removed with conventional treatment processes and must be accounted for to meet permit requirements. Tertiary treatment methods are likely needed to reliably achieve very low effluent TN concentrations below 2.5 to 3.0 mg/L if the ESON is above 1.5 mg/L. For wastewater effluents with much higher ESON concentrations, tertiary treatment may be needed to meet a TN of 3.0 mg/L.

●●●● BIOASSAYS FOR MEASURING SON

At present, there is no consensus as to the appropriate way to determine bSON or rSON using bioassays. Two possible approaches are outlined below.

What Are the Goals of SON Bioassays?

Bioassays are used to determine the biodegradability or bioavailability of SON. The portion of influent SON that can be removed by bacteria in the wastewater treatment process (biodegradable) and the portion of effluent SON that can be consumed by algae (bioavailable) in surface waters are of interest with regard to the fate of SON and its impact. The type of bioassay depends on the application and goal of the test. For in-plant issues the test goals include 1) determining if the treatment process was able to minimize the biodegradable SON (btSON), and 2) what portion of the SON in the influent wastewater and recycle streams is biodegradable or recalcitrant to treatment (btSON or rtSON). All of these goals involve the BNR treatment process and the biodegradability of SON by bacteria. Therefore, the bioassay procedure should incorporate biomass from the BNR process being assessed. This approach is referred to as a “technology-based bioassay” because it assesses the biodegradability of SON during the treatment process (Awobamise et al. 2007). For plant effluents the impact of ESON to surface water quality is of interest and the bioassay determines the bioavailability of ESON (bESON) to algae and thus its impact on eutrophication.

The results of the Nutrient Removal Challenge suggest that the fate of ESON in surface waters is affected by two fractions of ESON based on the hydrophobic and hydrophilic portions because the transformation of these two have different utilization rates by algae as shown in the Nutrient Removal Challenge Program study by Li et al. (2015). An ion exchange resin treatment method was developed to separate the fractions and the utilization rates of the each fraction was determined in bioassay tests.

What Is the Technology-based SON Bioassay Protocol Presently Used?

Khan (2007) used a technology-based assessment protocol (Table 8) to determine if the ESON could be lower by improving the biodegradation performance for SON in the activated sludge process. A longer detention time or SRT may be needed to minimize the btSON concentration. The test is done with 300 mL BOD bottles and follows changes in DO and SON concentrations with time. The btSON concentration is the difference between the initial SON concentration and the SON after exposure to biomass for a period of time. Because the method is a technology-based bioassay that looks at the potential for BNR mixed liquor to further biodegrade SON it is appropriate to conduct the assays in the dark because photosynthetic metabolisms do not routinely occur in activated sludge treatment. This bioassay may be used to evaluate the impact of various BNR process designs on minimizing btSON, the contribution and impact of recycle flows, and the potential for increasing the system SRT to further reduce the system ESON concentration.

Table 8 - Biodegradable (bESON) Bioassay Protocol (300 mL BOD Bottles)

Test Components	Procedure	Comments
Sample Preparation	Use filtrate from 0.22 µm glass fiber filtration ^a	Effluent filtrate or primary effluent.
	Saturate DO by aeration or shaking	
	Add 2 mL inoculum	Inoculum is mixed liquor from the same treatment plant at 240 mg/L MLSS
Seed Control	Add 2 mL inoculum to distilled water	
Test Bottle Incubation	300 mL bottle unmixed, 20°C	In the dark
	5-20 days ^a	
	Check and adjust DO periodically	Time intervals may be 0, 5, 10, 20 days
SON Measurements	Measure SON at sample time intervals	Time intervals may be at 0, 5, 10, 20 days

^aAwobamise et al. (2007) found most bESON to be gone by 20 to 30 days

Source: Awobamise et al. 2007

What Is the Goal of the Water Quality-based Bioassay?

A surface water quality-based assessment protocol under consideration is summarized in Table 9. It was first applied to measure bSON by Pehlivanoglu and Sedlak (2004) and later by Urgan-Demirtas et al. (2007) for a number of BNR effluents. In both cases, more SON was consumed when bacteria were present in the test with algae versus algae alone, indicating a synergistic relationship between algae and bacteria. The test uses a freshwater algae, thereby limiting its application to BNR plants that discharge into exclusively freshwater watersheds. Modifications to the protocol are done to determine the bSON or rSON for discharges located in watersheds that ultimately flow into estuarine water bodies that exist within estuarine watersheds (Mulholland et al. 2007). Modifications to this procedure were followed in the work by Li et al. (2015). The autoclaved the sample to inactivate indigenous algae and did not add bacteria inocula for concern of adding other algae. However, Sedlak et al. (2013) observed their incubated samples under a microscope to determine that no other algae were present besides their algae inocula.

Table 9 - Water Quality-based Assessment Protocol for Determining bSON
 (Using 500-mL Sample Flasks and 14-d Incubation)

Test Components	Procedure
Sample Preparation	1. Chlorinated effluent samples; dechlorinated with sulfur dioxide
	2. Use filtrate from 0.20 µm glass fiber filtration and fractionate with ultrafilters down to 1 kilodalton MW
	3. Distilled water and ESON samples spiked with 1 mg/L NO ₃ -N were run in parallel
Bacteria Inocula	1. Filter 3L of surface water first with 1 µm glass fiber filter
	2. Filter 1 µm filtrate through 0.20 µm membrane filter
	3. Suspend retentate of 0.20 µm membrane filter in 100 mL of 0.20 µm filtered surface water
	4. Add 1 mL of bacteria suspension to 400 mL sample
Algae Inocula	1. Use lab-cultivated freshwater algal, <i>Pseudokirchneriella subcapitata</i>
	2. Algae cultured per algae toxicity test protocol (APHA 2005), amended with nutrients except nitrate. K ₂ HPO ₄ added to media for a 3.0 N/P molar ratio.
	3. Add 5 mL of algal suspension at log growth to 400-mL sample
Test Flask Incubation	1. In shaker at 20-22° C
	2. 12-hr light/dark cycle
Algal Growth	Monitor with vivo chlorophyll-a measurements using fluorometer until stationary growth phase reached
SON Measurements	Measure SON at sample time intervals

Source: Pehlivanoglu and Sedlak 2004, Sedlak et al. 2013

The bSON consumed by the algae is predicted by measuring the algal chlorophyll production in sample bottles. A set of control sample bottles are spiked with nitrate to obtain a correlation between chlorophyll production and the amount of nitrogen consumed by the algae. A benefit of this method is that it is relatively easy to standardize and implement. If results from this method are found to correlate in a predictable way with more complex bioassays that use indigenous microbiota, then it could be valuable as an indicator.

What Factors Affect the Bioavailability of ESON in Surface Waters and Should Be Considered in the Surface Water Quality-based Assessment Protocol for Saline and Fresh Water Environment?

Key parameters that appear to affect the bioavailability of ESON by bacteria and algae include the salinity and pH of the water receiving ESON. It appears that nitrogen-containing humic substances are more bioavailable in saline water versus fresh water. The sorption of ammonium on humic material is also affected by salinity and ammonium is likely to desorb in higher salinity waters. In addition to physical and chemical interactions of nitrogen species due to water chemistry, it is known that water chemistry affects the populations of bacteria and algae species present in surface waters, which in turn results in different abilities for SON transformation. These variations in population dynamics across a receiving stream watershed are not captured in the previously mentioned protocols. Therefore, the ideal surface water quality-based assessment protocol should consider the receiving water quality and microbial diversity conditions present. Doing so with a protocol, however, complicates the method significantly beyond the other methods described here.

The soluble inorganic nitrogen ($\text{NH}_3\text{-N}$, $\text{NO}_3\text{-N}$, and $\text{NO}_2\text{-N}$) content of the sample may also affect the accuracy of bioassay protocols that involve use of algae and rely on measuring chlorophyll *a* production. High ratios of effluent inorganic nitrogen to SON will result in very high levels of chlorophyll produced from the inorganic nitrogen relative to chlorophyll produced by SON. It can be difficult to accurately quantify the amount of chlorophyll that actually was generated by the SON in a high background of DIN-generated chlorophyll, thereby compromising the method. To overcome this, DIN must be removed from or reduced in samples while retaining the SON.

RESEARCH NEEDS

As regulations require more stringent effluent nutrient concentrations to protect impaired surface waters from eutrophication, the impact of ESON has become more important and represents a new challenge in the area of biological nutrient removal. Initial efforts to measure ESON and its availability for bacteria and algae and to understand its removal in BNR treatment processes has led to both useful findings and an awareness of the need for more research on this topic. The research needs are summarized here for the topic areas raised in the Nutrient Removal Challenge.

Effluent SON concentration was found to be one of the most significant nitrogen species that limited the ability to achieve very low TN concentration with bioavailability in surface waters. In addition, program studies found that over 70 percent of effluent SON in full-scale BNR facilities was bioavailable for algae growth. It was also found that effluent SON concentrations were site-specific and varied over a wide range for different nutrient removal WRRFs. A better understanding of the sources of SON and its composition are needed.

Little has been done on the effect of aerobic and anaerobic digestion on SON concentrations and biosolids dewatering return flows. Further work is needed to explore innovative processes for effluent SON removal. Such work may involve a combination of different physical/chemical treatment methods.

Research is needed to develop more effective tertiary treatment technologies for ESON removal. A protocol for evaluating site-specific SON and its impact on effluent SON and meeting TN concentration limits need to be developed.

●●●● REFERENCES

- Asano, T., F. Burton, H. Leverenz, R. Tsuchihashi, and G. Tchobanoglous. 2006. *Water Reuse: Issues, Technologies, and Applications*. New York: McGraw-Hill.
- Awobamise, M., K. Jones, E. Khan, and S. Murthy. 2007. Long-Term Biodegradability of Dissolved Organic Nitrogen. In *Proceedings of the Water Environment Federation Technical Exposition and Conference (WEFTEC)*. San Diego, October 2007.
- Bott, C.B., and D.S. Parker. 2011. *Nutrient Management Volume II: Removal Technology Performance and Reliability*. Project NUTR1R06k/1512. Alexandria, VA: Water Environment Research Foundation, and London: IWA Publishing.
- Bratby, J., J. Jimenez, and D. Parker. 2008. Dissolved Organic Nitrogen—Is It Significant and Can It Be Removed? In *Proceedings of the Water Environment Federation Technical Exposition and Conference (WEFTEC)*. Chicago, Illinois, October 2008.
- Brett, M., and B. Li. 2015. *The Bioavailable Phosphorus (BAP) Fraction in Effluent from Advanced Secondary and Tertiary Treatment*. NUTR1R06m/1514. Alexandria, VA: Water Environment Research Foundation, and London: IWA Publishing.
- Mullholland, M.R., N.G. Love, D. A. Bronk, V. Pattarkine, A. Pramanik, and H.D. Stensel. 2009. "Fate and Transport of Organic N in Aquatic Systems." In *Establishing a Research Agenda for Assessing the Bioavailability of Wastewater Treatment Plant-Derived Effluent Organic Nitrogen in Treatment Systems and Receiving Waters*, 8–12. Publication 09-002. Edgewater, MD: Chesapeake Research Consortium, Inc. Scientific and Technical Advisory Committee (STAC). www.chesapeake.org/stac/Pubs/eonworkshopreport.pdf.
- Chen, B., Y. Kim, and P. Westerhoff. 2011. Occurrence and Treatment of Wastewater-Derived Organic Nitrogen. *Water Research*, 45(15), 4641-4650.
- Czerwionka, K., J. Makinia, K. Pagilla, and H. D. Stensel. 2012. Characteristics and Fate of Organic Nitrogen in Municipal Biological Nutrient Removal Wastewater Treatment Plants. *Water Research*, 46(7), 2057-2066.
- Czerwionka, K., and J. Makinia. 2014. Dissolved and Colloidal Organic Nitrogen Removal from Wastewater Treatment Plants Effluents and Reject Waters Using Physical-Chemical Processes. *Water Science & Technology*, 70(3), 561-568.
- Dwyer, J., L. Kavanagh, and P. Lant. 2008. The Degradation of Dissolved Organic Nitrogen Associated with Melanoidin Using a UV/H₂O₂ AOP. *Chemosphere*, 71(9), 1745-1753.
- Gu, A.Z., and N. Tooker. 2015. *Impact of Advanced Oxidation Processes on the Composition and Biodegradability of Soluble Organic Nutrients in Wastewater Effluents*. Project NUTR5R14e/1530. Alexandria, VA: Water Environment Research Foundation, and London: IWA Publishing.
- Jimenez, J., T. Madhanagopal, H. Schmidt, J. Bratby, H. Meka, and D. Parker. 2007a. Full-Scale Operation of Large Biological Nutrient Removal Facilities to Meet Limits of Technology Effluent Requirements: The Florida Experience. In *Proceedings of the Water Environment Federation Technical Exposition and Conference (WEFTEC)*. San Diego, October 2007.

- Jimenez, J., D. Parker, W. Zdziebloski, R. Pope, D. Phillips, J. Nissen, and H. Schmidt. 2007b. Achieving Limits of Technology (LOT) Effluent Nitrogen and Phosphorus Removal at the River Oaks Two-Stage Advanced Wastewater Treatment Plant. In *Proceedings of the Water Environment Federation Technical Exposition and Conference (WEFTEC)*. San Diego, October 2007.
- Karabacakoğlu, B., F. Tümsek, H. Demiral, and I. Demiral. 2008. Liquid Phase Adsorption of Phenol by Activated Carbon Derived from Hazelnut Bagasse. *Journal International Environmental Application & Science*, 3(5), 373-380.
- Khan, E. 2007. Development of Technology Based Biodegradable Dissolved Organic Nitrogen (BSON) Protocol. Presented at Water Environment Research Foundation and Chesapeake Bay Science and Technology Advisory Committee Workshop, Baltimore, MD, September 2007.
- Li, B., L. Fan, and M. Brett. 2015. *Mineralization Kinetics of Soluble Phosphorus and Soluble Organic Nitrogen in Advanced Nutrient Removal Effluents*. Project NUTR1R06p/1517. Alexandria, VA: Water Environment Research Foundation, and London: IWA Publishing.
- Liu, H., J. Jeong, H. Gray, S. Smith, and D. Sedlak. 2012. Algal Uptake of Hydrophobic and Hydrophilic Dissolved Organic Nitrogen in Effluent from Biological Nutrient Removal Municipal Wastewater Treatment Systems. *Environmental Science & Technology*, 46: 713-720.
- Merlo, R., J. Wong, V. Occiano, K. Sandera, A. Pai, S. Sen, J. Jimenez, D. Parker, and J. Burcham. 2012. Analysis of Organic Nitrogen Removal in Municipal Wastewater by Reverse Osmosis. *Water Environment Research*, 84(7), 588-595.
- Mulholland, M., N. Love, V. Pattarkine, D. Bronk, and E. Canuel. 2007. *Bioavailability of Organic Nitrogen from Treated Wastewater*. Report 07-001. Edgewater, MD: Chesapeake Research Consortium, Inc. Scientific and Technical Advisory Committee (STAC). www.chesapeake.org/stac/Pubs/OrganicNitrogenReport.pdf.
- Neethling, J.B., D. Clark, H.D. Stensel, J. Sandino, and R. Tsuchihashi. 2019. *Nutrient Removal Challenge Synthesis Report*. Project NUTR5R14g/4827g. Alexandria, VA: The Water Research Foundation.
- O'Shaughnessy, G., B. Harvey, J. Sizemore, and S. Murthy. 2006. Influence of Plant Parameters on Effluent Organic Nitrogen. In *Proceedings of the Water Environment Federation Technical Exposition and Conference (WEFTEC)*. Washington, DC, October 2006.
- Pagilla, K. 2007. Presentation at Water Environment Research Foundation and Chesapeake Bay Science and Technology Advisory Committee Workshop, Baltimore, MD, September 2007.
- Parkin, G., and P. McCarty. 1981. Sources of Soluble Organic Nitrogen in Activated Sludge Effluents. *Journal Water Pollution Control Federation*, 53(1).
- Pehlivanoglu, E., and D. Sedlak. 2004. Bioavailability of Wastewater-Derived Organic Nitrogen to the Alga *Selenasstrum Capricornutum*. *Water Research*, (38), 3189-3196.
- Pellegrin, M., Neethling, J.B., A. Menniti, J. Sandino, and D. Stensel. 2015. *Application of Membrane Bioreactor Processes for Achieving Low Effluent Nutrient Concentrations*. Project NUTR1R06u/1522. Alexandria, VA: Water Environment Research Foundation, and London: IWA Publishing.

- Qin, J., K. Kekre, G. Tao, M. Oo, M. Wai, T. Lee, B. Viswanath, and H. Seah. 2006. New Option of MBR-RO Process for Production of NEWater From Domestic Sewage. *Journal of Membrane Science*, 272(1), 70-77.
- Randtke, S., and P. McCarty. 1977. Variations of Nitrogen and Organics in Wastewater. *Journal Environmental Engineering Division*, 103(34), 539-550.
- Randtke, S., and P.L. McCarty. 1979. Removal of Soluble Secondary-Effluent Organics. *Journal of the Environmental Engineering Division*, 105(4), 727-743.
- Sedlak, D., and E. Pehlivanoglu. 2007. rSON Rate and Availability to Nitrogen-limited Algae. Presented at Water Environment Research Foundation and Chesapeake Bay Science and Technology Advisory Committee Workshop, Baltimore, MD, September 2007.
- Sedlak, D., J. Jeong, and H. Liu. 2013. *Uptake by Algae of Dissolved Organic Nitrogen from BNR Treatment Plant Effluents*. Project NUTR1R06e/1509. Alexandria, VA: Water Environment Research Foundation.
- Sharp, R., and J. Brown. 2007. Assessing Sources and Fate of rSON at Stamford, CT WPCF: Methods Development and Initial Results. Presented at Water Environment Research Foundation and Chesapeake Bay Science and Technology Advisory Committee Workshop, Baltimore, MD, September 2007.
- Shah, A., N. Dai, and W. Mitch. 2013. Application of Ultraviolet, Ozone, and Advanced Oxidation Treatments to Washwaters to Destroy Nitrosamines, Nitramines, Amines, and Aldehydes Formed During Amine-Based Carbon Capture. *Environmental Science & Technology*, 47(6), 2799-2808.
- Urgun-Demirtas, M., C. Sattayatewa, and K. Pagilla. 2008. Bioavailability of Dissolved Organic Nitrogen in Treated Effluents. *Water Environment Research*, 80(5), 397-406.
- Wikramanayake, R., G. Baker, E. Lawrence, D. St. Germain, S. Ong, J. Young, R. Martin, and D. Mozena. 2007. A Low Cost Solution to Reduce Total Nitrogen Discharged from WWTPs—Meeting the 3 mg/L Regulatory Limit in Total Nitrogen Using Existing Downflow Tertiary Media Filters as a Medium for Denitrification. In *Proceedings of the Water Environment Federation Technical Exposition and Conference (WEFTEC)*. San Diego, October 2007.