CHAPTER SIXTEEN

Protocol for the Measurement of Nitrous Oxide Fluxes from Biological Wastewater Treatment Plants

Kartik Chandran

Contents

Abstract

The overarching goal herein was to develop a protocol that could be used to generate consistent information on the generation and emission of nitrous oxide (N_2O) from open-surface wastewater treatment bioreactors. The developed protocol was reviewed and endorsed by the United States Environmental Protection Agency (USEPA), whereupon it was used to determine N_2O emissions

Department of Earth and Environmental Engineering, Columbia University, New York, USA

Methods in Enzymology, Volume 486
ISSN 0076-6879, DOI: 10.1016/S0076-6879(11)86016-7

from a wide array of wastewater treatment processes across the United States. Scaled-down variants of the protocol have also since been adopted for lab-scale measurements. The protocol consists of a combination of elements that entail real-time online measurement of headspace N_2O concentrations, supplemented by discrete measurements of liquid-phase N_2O and other routinely monitored wastewater and process parameters. Notably, the advective flow rate of headspace gas is also directly measured.

1. Introduction

Based on recent field-scale measurements, engineered biological nitrogen removal (BNR) plants, while effective to varying degrees in reducing *aqueous* nitrogen pollution could emit up to 7% of the influent nitrogen load as *gaseous* nitrous oxide (N_2O) and nitric oxide (NO) (Kampschreur *et al.*, 2008). Such emissions are deleterious to the environment. The greenhouse equivalence of N_2O is about 300 times that of carbon dioxide and both N_2O and NO contribute to depletion of the ozone layer (Ravishankara *et al.*, 2009). From a biological perspective, N_2O and NO are known intermediates in heterotrophic denitrification (Knowles, 1982; Zumft, 1997) and autotrophic nitrification and denitrification (Anderson and Levine, 1986; Anderson *et al.*, 1993; Kester *et al.*, 1997; Ritchie and Nicholas, 1972; Stuven *et al.*, 1992). However, the net contribution of processes such as denitrification wastewater treatment plants (WWTPs) to N_2O emissions has only recently been explicitly acknowledged (USEPA, 2009). Additionally, there is a real paucity of systematic protocols that enable collection of N_2O emission fluxes from open-surface activated sludge bioreactors using consistent methodology. The development and application of a detailed protocol to conduct plant-wide measurements of gaseous and aqueous N_2O concentrations is described herein. This protocol is intended to provide utilities and field sampling teams with a detailed description of the data collection methodology and analysis requirements to enable calculation of gaseous nitrogen fluxes from different zones of activated sludge trains in a wastewater treatment facility. The protocol was reviewed and endorsed by the United States Environmental Protection Agency (USEPA) during Fall 2008 and has since been implemented at different WWTPs in North America toward the quantification of N_2O emissions therein as described elsewhere (Ahn *et al.*, 2010a,b).

2. Sampling Design for Full-Scale Monitoring

The N_2O emission fluxes of several BNR and non-BNR WWTPs were measured (Table 16.1). Testing was conducted at each plant during which gas-phase monitoring was performed in real-time continuous mode

Table 16.1 Summary of process schematics sampled

Plant configuration	Description
Separate-stage BNR	The low-rate separate-stage nitrification denitrification process at this WWTP was sampled. The process was configured as a sequence of five reactors in series. The influent to this process consisted of the clarified effluent from an upstream high-rate process, mainly engaged organic carbon removal. The influent was fed in a step-feed fashion to the first two aerobic zones. The last three zones of this process were nonaerated and the second nonaerated zone received methanol to promote denitrification. The effluent channel of this process was aerated prior to secondary clarification.
Four-stage Bardenpho	The four-stage Bardenpho process consisted of predenitrification (without external carbon addition) followed by a primary aerated zone. The effluent of the primary aerated zone was internally recycled to the anoxic zone. Following the primary aerated zone was a deoxygenation zone to scavenge dissolved oxygen, prior to methanol addition for enhanced denitrification. The final zone in this process was aerated primarily for stripping off the dinitrogen gas produced during denitrification, prior to secondary clarification.
Step-feed BNR 1	The four-pass step-feed BNR process sampled consisted of preanoxic zones comprising about 1/3 of the pass volume followed by aerated zones. The transition zone between each pass was nonaerated to facilitate deoxygenation. The approximate influent flow split was 10–40–30–20% to the four passes, respectively. The first pass also received presettled anaerobic digestion centrate, which constituted approximately 30% of the influent TKN load to the process. Return activated sludge was also fed to the first pass.
Step-feed non-BNR	The step-feed non-BNR process sampled was configured and operated in four-pass step-aeration mode. The process was completely covered primarily for odor control. The headspace off-gases were consolidated and fed to a biofilter. The approximate influent flow split was 10–40–30–20% to the four passes, respectively. Return activated sludge was fed to the first pass.
Separate centrate	The separate centrate treatment process was operated to process presettled anaerobic digestion centrate and partially convert the influent NH_4^+–N to NO_2^-–N. The separate centrate treatment process was operated in plug-flow mode. Effluent from the separate centrate tank was fed to the overall plant return activated sludge line for possible bioaugmentation with primarily ammonia oxidizing bacteria (AOB) and for nitrogen removal via the short-cut nitrite pathway similar to that described in (van Dongen *et al.*, 2001).
Plug-flow 1	The first plug-flow process sampled was designed and operated primarily for organic carbon removal and nitrification and did not have dedicated anoxic zones or external organic carbon addition. The process was configured in four-pass mode.

(*continued*)

Table 16.1 (*continued*)

Plant configuration	Description
Plug-flow 2	The second plug-flow process sampled was also designed and operated for organic carbon removal and nitrification and did not have dedicated anoxic zones or external organic carbon addition. The process was configured in two-pass mode.
MLE 1	The first modified Lutzack Ettinger (MLE) process sampled was originally designed for operation in enhanced biological phosphorous removal mode, but subsequently operated in MLE mode. The process consisted of predenitrification without external organic carbon addition.
MLE 2	The second modified Lutzack Ettinger (MLE) process sampled was also originally designed for operation in enhanced biological phosphorous removal mode, but subsequently operated in MLE mode. The process consisted of predenitrification without external organic carbon addition.
Step-feed BNR 2	The second step-feed process sampled was configured in four-pass mode. Each pass consisted of preanoxic zones comprising 1/3 of the pass volume followed by aerobic zones. The approximate influent flow split was 50–30–20–0% to the four passes, respectively. The anoxic zones were mixed via low intensity pulse aeration. The return activated sludge was fed to the first pass.
Oxidation ditch	The oxidation ditch process was operated to achieve simultaneous nitrification and denitrification by operation at uniformly low aeration intensities and dissolved oxygen concentrations. The influent flow to the process was fed to the inner loop and was mixed and circulated using surface mixers. No external organic carbon was added to enhance denitrification. Return activated sludge was fed to the inner loop of the process.
Step-feed BNR 3	The third four-pass step-feed BNR process sampled consisted of preanoxic zones comprising about 1/3 of the pass volume followed by aerated zones. The approximate influent flow split was 33.3–33.3–33.3–0% to the four passes, respectively. The first pass also received presettled anaerobic digestion centrate, which constituted approximately 40% of the influent TKN load to the process. Return activated sludge was also fed to the first pass. The reactors of this process were also covered and thus only composite measurements of the overall headspace could be performed.

and liquid-phase sampling was performed via a combination of plant online analyzers (where available) and discrete grab sampling conducted by plant operators and laboratory staff. The wastewater and process analytes sampled and the frequency and location of sampling at a typical WWTP are detailed in Table 16.2.

3. Sampling Procedures: Headspace Gas Measurement

The overall procedure for measuring N_2O, NO, and NO_2 fluxes from the headspace of activated sludge tanks involved a variant of the EPA/600/8-86/008 and the South Coast Air Quality Management District (SCAQMD) tracer methods. This variant was developed to measure those sources that have a relatively high surface flux rate when compared to diffusion (for instance, WWTPs). Commercially available replicas of the US EPA surface emission isolation flux chamber (SEIFC; Figs. 16.1 and 16.2) were used to measure gaseous N fluxes from activated sludge reactors. The SEIFC consisted of a floating enclosed space from which exhaust gas was collected in a real-time or discrete fashion. Since the surface area under the SEIFC could be measured, the specific flux of the gaseous compound of interest could be determined. The SEIFC "floated" on the activated sludge tank surface (Fig. 16.1, right panel) and several replicate measurements could be obtained at different locations in a single tank as well as from different tanks (nitrification, denitrification) along a treatment train. The SEIFC was also equipped with mixing via sweep gas circulation to ensure collection of representative gas-phase concentrations. The SEIFC is currently one of the few devices accepted by the USEPA for measuring gaseous fluxes (Tata *et al.*, 2003).

Sampling was conducted at multiple locations of the activated sludge train in each wastewater treatment facility. These specific locations selected were the geometric center of each demarcated anoxic or aerobic zone in the WWTP, or alternately locations where nitrification could be inferred based on initial screening of NH_4^+–N and DO concentrations (as in the plug-flow processes). For discrete measurement at each of these locations, 30 replicate measurements of gaseous N_2O and one measurement of aqueous N_2O were obtained over a period of 30 min. During continuous measurement at each of these specific locations over a 24-h period, gaseous N_2O concentrations were still measured once per minute, while aqueous N_2O concentrations were measured about six times per day. Independent replication at each location (on different days) was not conducted owing to practical limitations associated with such an extensive campaign.

Table 16.2 Typical wastewater and process measurements conducted in parallel with gas-phase monitoring

	Analyte													
Sample location	TSS	VSS	Total $cBOD_5$	Soluble $cBOD_5$	Total COD	Sol. COD	ff COD	TKN	Sol. TKN	pH	Alk	NH_3–N	NO_3–N	NO_2–N
Primary effluent	8/d	2/d	8/d	8/d	8/d	8/d	8/d	8/d	8/d	8/d	8/d	8/d	8/d	8/d
Secondary effluent	8/d	–	8/d	8/d	8/d	8/d	8/d	8/d	8/d	8/d	8/d	8/d	8/d	8/d
RAS MLSS	8/d													
WAS MLSS	8/d													
Operating data														
Influent flow	Diurnal flow pattern at appropriate time intervals (15 min for periods of rapid diurnal increase, 1 h for stable periods)													
RAS flow	Average daily RAS flow, Indicate location and type of flow measurement and variability of flow													
WAS flow	Average daily WAS flow, Indicate location and type of flow measurement, times of WAS wasting if not continuous													
Dissolved oxygen	1 h^{-1}, indicate location of DO measurement along basin length and time of measurement													
Aeration rate	Daily average, indicate location of Air Flow Measurement and variability over the course of the day. SCADA output at short time intervals would be best													
In-tank profiles	TSS		VSS	pH	DO	ORP	Temp.	ff COD		Alk.	NH_3–N		NO_3–N	NO_2–N
	8/d		2/d	8/d	8/d	8/d	8/d	8/d		8/d	8/d		8/d	8/d

TSS, total suspended solids; VSS, volatile suspended solids; $cBOD_5$, carbonaceous 5-day biological oxygen demand; COD, chemical oxygen demand; Sol COD, soluble chemical oxygen demand; ffCOD, filtered flocculated chemical oxygen demand (as described by Mamais *et al.*, 1993); TKN, total Kjeldahl nitrogen; Alk, alkalinity; NH_3–N, ammoniacal nitrogen; NO_3–N, nitrate nitrogen; NO_2–N, nitrite nitrogen; RAS, return activated sludge; MLSS, mixed liquor suspended solids; WAS, waste activated sludge; DO, dissolved oxygen; ORP, oxidation-reduction potential.

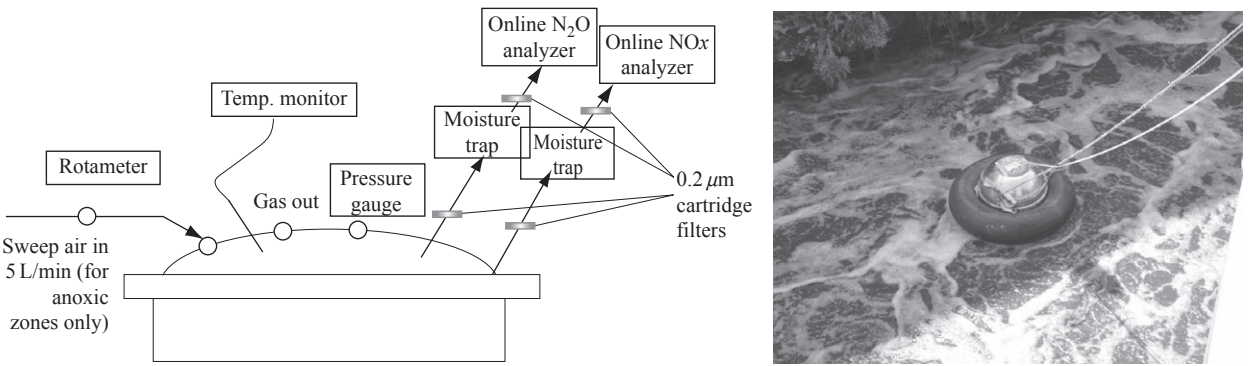

Figure 16.1 Schematic of SEIFC (left panel) and SEIFC deployment at a full-scale WWTP for N_2O measurement (right panel).

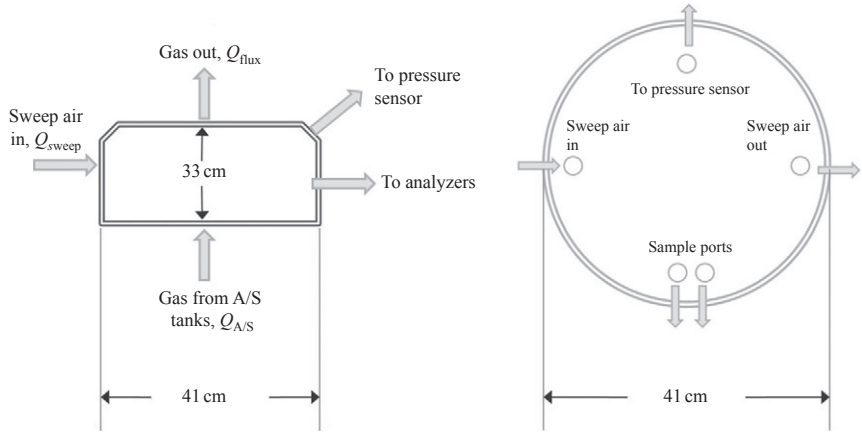

Figure 16.2 Schematic of gas flows in and out of the flux chamber in elevation (left panel) and plan (right panel) views.

4. Sampling Procedures: Measurement of Aqueous N_2O Concentrations

Aqueous phase N_2O concentrations were measured using a miniaturized Clark-type sensor with an internal reference and a guard cathode (Unisense, Aarhus, Denmark). The sensor was equipped with an oxygen front guard, which prevented oxygen from interfering with the nitrous oxide measurements. The sensor was coupled to a high-sensitivity picoammeter to convert the current resulting from cathodic reduction of N_2O to an electric signal. Aqueous N_2O measurements were conducted right adjacent to the SEIFC location about six times per day, coincident with other liquid-phase wastewater measurements, which were indicative of the performance of the treatment plant.

5. Sampling Procedures: Measurement of Advective Gas Flow Rate from Bioreactor Headspace

One of the most important developments included in this protocol is the explicit measurement of the advective flow of gases through the flux chamber. By measuring the gas flow rate, the actual "flux" of N_2O can be thus computed (as discussed below). Advective flow of gas through the flux chamber ($Q_{emission}$) *in aerated zones* was measured using a modification of American Society for Testing and Materials (ASTM) method D1946. Briefly, a tracer gas consisting of 100,000 ppmv ($C_{helium-tracer}$) He was introduced

into the flux chamber at a known flow rate, Q_{tracer} (Eq. (16.1)). He concentrations in the off-gas from the flux chamber ($C_{helium-FC}$) were measured using a field gas-chromatograph equipped with a thermal conductivity detector (GC–TCD). $Q_{emission}$ was computed using Eq. (16.2).

$$Q_{tracer} \times C_{helium-tracer} = (Q_{tracer} + Q_{emission}) \times C_{helium-FC}. \quad (16.1)$$

$$Q_{emission} = \frac{Q_{tracer} \times (C_{helium-tracer} - C_{helium-FC})}{C_{helium-FC}}. \quad (16.2)$$

The only modification to the protocol to measure the emission flow rate from *nonaerated zones* was the introduction of sweep gas (air) or carrier gas through the headspace of the flux chamber at a known flow rate ($Q_{sweep} = 5$ L/min), in addition to the He tracer gas (Eq. (16.3)). The corresponding $Q_{emission}$ was computed using Eq. (16.4). Addition of sweep gas is needed to promote mixing of the SEIFC contents, owing to the low advective gas flow from the anoxic-zone headspace. Sweep-air N_2O concentrations were always measured and typically below the detection limits of the N_2O analyzer.

$$Q_{tracer} \times C_{helium-tracer} = \left(Q_{tracer} + Q_{sweep} + Q_{emission}\right) \times C_{helium-FC}. \quad (16.3)$$

$$Q_{emission} = \frac{Q_{tracer} \times (C_{helium-tracer} - C_{helium-FC})}{C_{helium-FC}} - Q_{sweep}. \quad (16.4)$$

During continuous N_2O measurements, $Q_{emission}$ was determined several times a day to match liquid-phase N_2O measurements.

6. Principles of Real-Time N_2O Measurement

Continuous N_2O measurements were performed via infra-red (IR) gas-filter correlation (Teledyne API Model 320E, San Diego, CA), which is based on the absorption of IR radiation by N_2O molecules at wavelengths near 4.5 μm.

7. Data Analysis: Determination of Fluxes

The net flux of gaseous N species (mg/min-m^2) was calculated based on the gas flow rate out of the flux chamber ($Q_{emission}$, L/min), headspace gas concentration (parts per million volume) and the cross-sectional area of the SEIFC (m^2) (Eq. (16.5)).

$$\text{Flux} = \frac{Q_{\text{emission}} \times C}{A}. \tag{16.5}$$

8. Data Analysis: Determination of Emission Fractions

The surface flux calculated from Eq. (16.5) was translated into the flux of a given zone by multiplying with the area of the specific zone in the wastewater treatment reactor, where the measurements were conducted. The N_2O emission fractions (mass/mass) for each WWTP at any given time point were computed by normalizing the measured flux from each zone in the facility to the daily influent total Kjeldahl nitrogen (TKN) loading according to Eq. (16.6). Emission fractions were averaged over the course of the diurnal sampling period and reported as the average (avg.) ± standard deviation (sd.) for each individual process sampled.

During each campaign, wastewater nitrogen species concentrations including influent, bioreactor and effluent TKN, ammonium, nitrite, and nitrate were measured simultaneously at least six times per day according to Standard Methods (Eaton *et al.*, 2005) to supplement the gas-phase measurements. The discrete measurements were averaged to generate the emission fractions described in Eq. (16.6). Additionally, seven out of the twelve processes (Table 16.1) were sampled at minimum and maximum annual wastewater temperatures to examine seasonal temperature impacts on N_2O generation and emission.

$$\text{Emission fraction} = \frac{\sum_{i=1}^{n} \text{Flux}_i \times \text{Area}_i (\text{kg}\,N_2O - N)}{\text{Daily influent TKN load } (\text{kg} - N)}, \tag{16.6}$$

where Flux_i, N_2O emission flux calculated from the ith zone (kg N_2O–N/m^2 d); Area_i, surface area of the ith zone (m^2); n, number of zones in a given facility from which N_2O fluxes are captured; Daily influent TKN load, average influent load (influent flow rate × influent TKN concentrations) over the course of 24 h.

9. Data Analysis: Calculation of N_2O Emission Factors

N_2O emission factors were computed by normalizing the total reactor N_2O mass flux to the unit population equivalent flow rate (100 gal/PE/day for the United States; Tchobanoglous *et al.*, 2003) and were expressed in

units consistent with the USEPA inventory report (g N_2O/PE/year) (USEPA, 2009). For aerobic zones, the helium-based advective gas-flow data were correlated to plant-recorded airflow rates for any given zone via linear regression and used to calculate diurnal N_2O emission factors. For anoxic (nonaerated) zones lacking associated plant airflow data, the average of the experimentally obtained helium-based gas flow rates was used to calculate diurnal N_2O emission factors.

10. Standardization of Protocol and Comparison with Established Emissions Flux Measurement Methods

The validity of the measurements using the protocol developed for this study was determined via a parallel sampling effort between two independent teams on September 9 and 10, 2008 at the wastewater treatment facility employing the step-feed BNR process 2 (Table 16.1). The Columbia University–Water Environment Research Foundation team (labeled WERF) used a flux chamber manufactured by St. Croix Sensory and measured N_2O off-gas concentrations via gas-filter correlation, described above. A second team (labeled CES) used an USEPA flux chamber and sampled the off-gas into opaque Tedlar® bags for subsequent Fourier-transform infra-red (FTIR) analysis (NIOSH 6660) by a commercial laboratory (Peak Analytical, Boulder, CO).

The possibility of "biasing" the measured N_2O concentrations by introduction of different sweep gas flow rates was also part of the validation testing. The successive-dilution method employed by the WERF team involved dilution of measured N_2O concentrations by virtue of introducing sweep gas at two different flow rates (4 and 8 L/min). To compare, the CES team employed ASTM method D1946, which involved introducing He tracer at 5 L/min. The equivalence of these two methods was determined by computing the headspace advective flow rate from the nonaerated zones.

Based on these parallel measurements conducted independently, similar results were obtained, with good correspondence in general in both the nitrous oxide fluxes (Fig. 16.3) and off-gas flow-rate (Fig. 16.4) in different zones of the selected activated sludge tank. The equivalence in the flow rates obtained using the two methods (successive dilution and He tracer) also helped to reject the possibility of "biasing" the measured N_2O concentrations due to changing hydrodynamic flow patterns in the headspace of the flux chamber by the introduction of sweep gas. Additionally, the following observations were made based on the results obtained and incorporated into subsequent full-scale measurement campaigns:

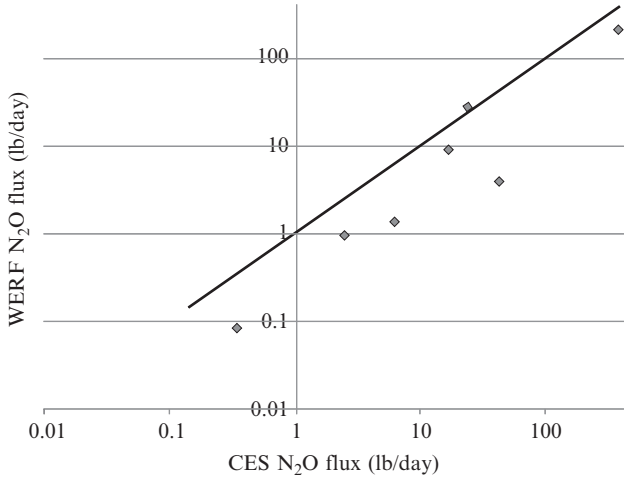

Figure 16.3 Comparison between N_2O fluxes obtained via two independent methods across eight zones of the four-pass step-feed BNR reactor 2.

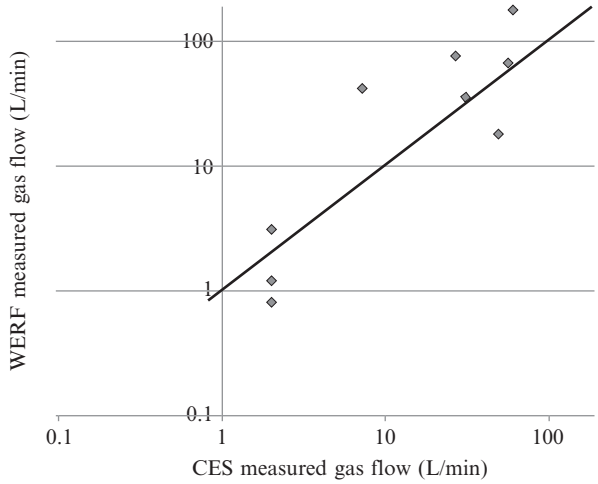

Figure 16.4 Comparison between gas flow rates obtained via the successive dilution (WERF) and tracer gas (CES) methods conducted at the step-feed BNR reactor 2.

a. The use of an inert gas tracer by the CES team was demonstrated to be an appropriate method to determine the advective off-gas flow rate. This was an operationally more facile and reliable method compared to the successive-dilution method developed by the Columbia University–WERF team based on successive dilution of the N_2O concentrations.

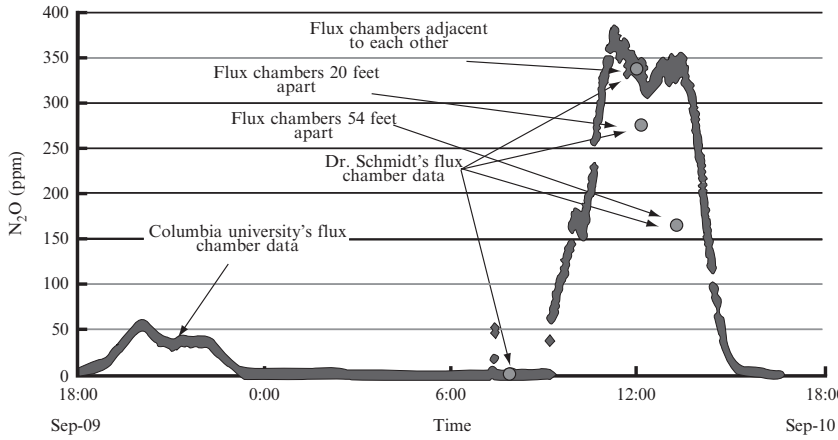

Figure 16.5 Illustration of spatial and temporal variability in N_2O concentrations in the headspace of an aerobic zone that necessitates real-time online monitoring. Columbia University–WERF's flux chamber data given by near continuous blue symbols, CES flux chamber data given by four discrete dots, as marked. Measurements were conducted at the step-feed BNR reactor 2 (courtesy, Dr. Charles E Schmidt).

Therefore, the successive-dilution method was discontinued following the parallel sampling study and replaced with He tracer-based method to determine advective flow rate.

b. Significant spatial and temporal variability in the measured concentrations of headspace N_2O was observed by the Columbia and the CES teams (Fig. 16.5). Therefore, for subsequent full-scale measurements, discrete measurements (initially proposed at a frequency of once a day) of N_2O at different locations in any given WWTP was discontinued. Instead, a significantly more involved sampling strategy that entailed 24 h "real-time online monitoring" of emissions at each location was initiated.

11. N_2O Emission Fluxes from Activated Sludge Processes

A wide range of N_2O emissions was measured across the 12 WWTPs operated at different temperatures, configurations, and influent characteristics. On average, N_2O emission fractions varied from 0.01% to 1.8% or 0.01% to 3.3%, when normalized to influent TKN load or influent TKN load processed, respectively (Ahn *et al.*, 2010b). These emission fractions were on the lower end of the range reported by previous studies, which

varied between 0% and 15% of influent TKN load (Czepiel *et al.*, 1995; Kampschreur *et al.*, 2008; Kimochi *et al.*, 1998; Sommer *et al.*, 1998; Sümer *et al.*, 1995; Wicht and Beier, 1995).

Computed flow-normalized emission factors also varied in a wide range, over two orders of magnitude (Ahn *et al.*, 2010b), and were mostly statistically higher (at the $\alpha = 0.05$ confidence level) than currently used values of 3.2 g N_2O/PE/yr (non-BNR processes; Czepiel *et al.*, 1995) or 7.0 g N_2O/PE/yr (BNR processes; USEPA, 2009). A high degree of diurnal variability in emission factors was also observed and could be linked diurnal variations in influent N-loading as reported by Ahn *et al.* (2010a). Based on the observed variability either diurnally or across the range of WWTPs sampled, the use of a "single" universal emission factor to calculate N_2O emissions from all wastewater treatment processes is inadequate.

In general, N_2O emissions in aerated zones were higher than those in nonaerated zones (Ahn *et al.*, 2010b). Therefore the currently held premise that N_2O emissions from WWTPs mostly occur in the anoxic zones (USEPA, 2009) is not accurate. Possible mechanisms for N_2O emissions via nitrification and denitrification have also been recently published (Lu and Chandran, 2010; Yu and Chandran, 2010; Yu *et al.*, 2010). Both processes likely contributed to the measured N_2O emissions. Good correlation in general was also obtained between liquid-phase and gaseous-phase N_2O concentrations as discussed in Ahn *et al.* (2010a). However, the due to possible interference with dissolved oxygen and nitric oxide, a high level of confidence could not be placed in the aqueous N_2O concentrations even at lab-scale (Yu *et al.*, 2010). Therefore, it is suggested that aqueous N_2O concentrations be alternately approximated based on estimated system-specific gas-liquid mass transfer coefficients, as described elsewhere (Yu *et al.*, 2010).

12. Triggers for N_2O Emission from Wastewater Treatment Operations

The data obtained during the national survey were subjected to multivariate data mining to identify potential triggers for N_2O emission from wastewater treatment operations, described elsewhere (Ahn *et al.*, 2010b). Based on this data mining approach, the triggers for N_2O emissions from aerobic zones were NH_4^+–N, NO_2^-–N, and DO concentrations in isolation and NH_4^+–N and NO_2^-–N concentrations in combination (Ahn *et al.*, 2010b). However, high DO and NO_2^-–N concentrations were positively correlated with N_2O emissions from anoxic zones (Ahn *et al.*, 2010b). For more details on the plant specific data and data mining, please consult (Ahn *et al.*, 2010b).

13. Lab-Scale and Field-Scale Adaptation of Protocol N_2O Emission Measurements

Although the original version of the protocol was developed for full-scale measurements of N_2O emissions from operational WWTPs, we have since successfully scaled down the protocol for lab-scale measurements. The scaled-down versions essentially employ smaller versions of the SEIFC to fit lab-scale bioreactors. Gas-phase and liquid-phase measurements are conducted in almost identical fashion to the full-scale version. The only difference is that the sampling frequency of liquid-phase variables, which is tailored depending on the experimental design. Using the scaled-down protocol, the magnitude and some novel mechanisms of N_2O emissions from both nitrifying and denitrifying lab-scale bioreactors have been recently reported (Lu and Chandran, 2010; Yu and Chandran, 2010; Yu *et al.*, 2010).

The protocol has also been shared with research groups in Belgium, Portugal, and Spain to facilitate similar full-scale N_2O measurement campaigns. Efforts at training wastewater treatment operators in the implementation of the protocol at several additional plants in the United States are also underway.

14. Concluding Remarks

A protocol to measure N_2O emission fluxes from WWTPs was developed. This protocol represents the first systematic attempt to develop a consistent methodology for the measurement of such emissions. As the focus of the wastewater industry shifts increasingly toward environmentally sustainable treatment, the measurement of the overall greenhouse gas footprint of such treatment processes becomes more relevant. Broad application of this protocol will thus enable WWTPs to quantify their N_2O emissions and engineer approaches that are aimed at minimizing both aqueous and gaseous nitrogen pollution.

ACKNOWLEDGMENT

This protocol was developed as part of a larger study to develop a national database of N_2O emissions from WWTP, funded by the Water Environment Research Foundation (WERF) Climate Change Program.

REFERENCES

Ahn, J.-H., Kim, S., Pagilla, K., Katehis, D., and Chandran, K. (2010a). Spatial and Temporal Variability in Atmospheric Nitrous Oxide Generation and Emission from Full-Scale Biological Nitrogen Removal and Non-BNR Processes. *Water Environ. Res.* **82**(10), DOI: 10.2175/106143010X12681059116897.

Ahn, J. H., Kim, S., Park, H., Rahm, B., Pagilla, K., and Chandran, K. (2010b). N_2O Emissions from activated sludge processes, 2008–2009: Results of a national monitoring survey in the United States. *Environ. Sci. Technol.* **44,** 4505–4511.

Anderson, I. C., and Levine, J. S. (1986). Relative rates of nitric oxide and nitrous oxide production by nitrifiers, denitrifiers and nitrate respirers. *Appl. Environ. Microbiol.* **51,** 938–945.

Anderson, I. C., Poth, M., Homstead, J., and Burdige, D. (1993). A comparison of NO and N_2O production by the autotrophic nitrifier *Nitrosomonas europaea* and the heterotrophic nitrifier *Alcaligenes faecalis*. *Appl. Environ. Microbiol.* **59,** 3525–3533.

Czepiel, P., Crill, P., and Harriss, R. (1995). Nitrous oxide emissions from municipal wastewater treatment. *Environ. Sci. Technol.* **29,** 2352–2356.

Eaton, A. D., Clesceri, L. S., and Greenberg, A. E. (eds.), (2005). Standard Methods for the Examination of Water and Wastewater, APHA, AWWA, and WEF, Washington DC.

Kampschreur, M. J., van der Star, W. R. L., Wielders, H. A., Mulder, J. W., Jetten, M. S. M., and van Loosdrecht, M. C. M. (2008). Dynamics of nitric oxide and nitrous oxide emission during full-scale reject water treatment. *Water Res.* **42,** 812–826.

Kester, R. A., de Boer, W., and Laanbroek, H. J. (1997). Production of NO and N_2O by pure cultures of nitrifying and denitrifying bacteria during changes in aeration. *Appl. Environ. Microbiol.* **63,** 3872–3877.

Kimochi, Y., Inamori, Y., Mizuochi, M., Xu, K.-Q., and Matsumura, M. (1998). Nitrogen removal and N_2O emission in a full-scale domestic wastewater treatment plant with intermittent aeration. *J. Ferment. Bioeng.* **86,** 202–206.

Knowles, R. (1982). Denitrification. *Microbiol. Rev.* **46,** 43–70.

Lu, H., and Chandran, K. (2010). Factors promoting emissions of nitrous oxide and nitric oxide from denitrifying sequencing batch reactors operated with methanol and ethanol as electron donors. *Biotechnol. Bioeng.* **106,** 390–398.

Mamais, D., Jenkins, D., and Pitt, P. (1993). A rapid physical-chemical for the determination of readily biodegradable soluble COD in municipal wastewater. *Water Res.* **27,** 195.

Ravishankara, A. R., Daniel, J. S., and Portmann, R. W. (2009). Nitrous Oxide (N_2O): The dominant ozone-depleting substance emitted in the 21st century. *Science* **326,** 123–125.

Ritchie, G. A. F., and Nicholas, D. J. D. (1972). Identification of the sources of nitrous oxide produced by oxidative and reductive processes in *Nitrosomonas europaea*. *Biochem. J.* **126,** 1181–1191.

Sommer, J., Ciplak, A., Sumer, E., Benckiser, G., and Ottow, J. C. G. (1998). Quantification of emitted and retained N_2O in a municipal wastewater treatment plant with activated sludge and nitrification-denitrification units. *Agrobiol. Res.* **51,** 59–73.

Stuven, R., Vollmer, M., and Bock, E. (1992). The impact of organic matter on nitric oxide formation by *Nitrosomonas europaea*. *Arch. Microbiol.* **158,** 439–443.

Sümer, E., Weiske, A., Benckiser, G., and Ottow, J. C. G. (1995). Influence of environmental conditions on the amount of N_2O released from activated sludge in a domestic waste water treatment plant. *Cell. Mol. Life Sci.* **51,** 419–422.

Tata, P., Witherspoon, J., and Lue-Hing, C. (eds.), (2003). VOC Emissions from Wastewater Treatment Plants, Lewis Publishers, Boca Raton, FL.

Tchobanoglous, G., Burton, F. L., and Stensel, H. D. (2003). Metcalf and Eddy Wastewater Engineering: Treatment and Reuse McGraw Hill, New York, NY.

USEPA (2009). Inventory of U.S. Greenhouse Gas Emissions and Sinks: 1990-2006, EPA 430-R-08-005 (Washington DC).

van Dongen, U., Jetten, M. S. M., and van Loosdrecht, M. C. M. (2001). The SHARON-ANAMMOX process for treatment of ammonium rich wastewater. *Water Sci. Technol.* **44,** 153–160.

Wicht, H., and Beier, M. (1995). N_2O emission aus nitrifizierenden und denitrificierended Klaranlagen. *Korresp. Abwasser* **42**(404–406), 411–413.

Yu, R., and Chandran, K. (2010). Strategies of *Nitrosomonas europaea* 19718 to counter low dissolved oxygen and high nitrite concentrations. *BMC Microbiol.* **10,** 70.

Yu, R., Kampschreur, M. J., van Loosdrecht, M. C. M., and Chandran, K. (2010). Mechanisms and specific directionality of autotrophic nitrous oxide and nitric oxide generation during transient anoxia. *Environ. Sci. Technol.* **44,** 1313–1319.

Zumft, W. G. (1997). Cell biology and molecular basis of denitrification. *Microbiol. Mol. Biol. Rev.* **61,** 533–616.