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Full-scale post denitrifying biofilters: sinks of dissolved N_2O?

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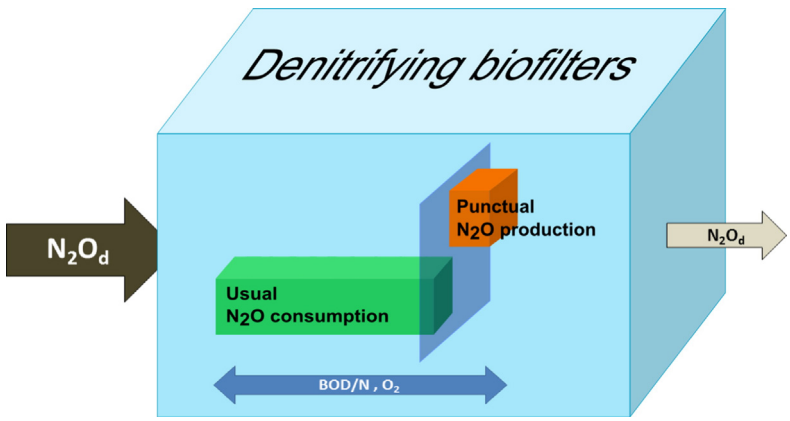
HIGHLIGHTS

- Monitoring of N_2O emissions for a full-scale denitrifying biofilter treatment plant.
- Most of N_2O remains in the liquid phase and is discharged with the effluent.
- Denitrification reduces part of dissolved N_2O produced during nitrification.
- N_2O peak emissions were related to severe carbon underdosing.

GRAPHICAL ABSTRACT



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ABSTRACT

In this study, nitrous oxide (N_2O) emissions from a full-scale denitrifying biofilter plant were continuously monitored over two periods (summer campaign in September 2014 and winter campaign in February 2015). Results of the summer campaign showed that the major part (>99%) of N_2O flux was found in the liquid phase and was discharged with the effluent. N_2O emissions were highly variable and represented in average 1.28 ± 1.99% and 0.22 ± 0.31% of the nitrate uptake rate during summer and winter campaigns, respectively. Denitrification was able to consume a large amount of dissolved N_2O coming from the upstream nitrification stage. In the absence of methanol injection failure and with an influent BOD/NO_3-N ratio higher than 3, average reduction of N_2O was estimated to be of 93%. The control of exogenous carbon dosage is essential to minimize N_2O production from denitrifying biofilters, in correlation to NO_2-N concentrations in the filter.

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1. Introduction

N_2O is a potent greenhouse gas with a global warming potential that is 265 times higher than carbon dioxide on a 100-years horizon and is also a primary ozone-depleting substance in the 21st century (Ravishankara et al., 2009; IPCC, 2013). In wastewater treatment

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processes, nitrous oxide is recognized to be emitted during nitrification and denitrification processes (Kampschreur et al., 2009; Law et al., 2012). In conventional wastewater treatment processes, heterotrophic denitrification reduces nitrate (NO_3^-) -generated from the autotrophic nitrification- to nitrogen gas (N_2) via nitrite (NO_2^-), nitric oxide (NO) and nitrous oxide (N_2O). This implies the action of four different denitrification reductases, namely nitrate reductase, nitrite reductase, NO reductase and N_2O reductase. Depending on the organisms involved and on the environmental conditions, the final product of heterotrophic denitrification is not always N_2, but could be in the form of undesirable intermediates such as nitrous oxide.

During heterotrophic denitrification, N_2O accumulation is thought to result from an unbalance of its production and consumption rates. Several parameters affecting the activity of N_2O reductase enzyme and correlated to N_2O accumulation were identified. They include dissolved oxygen, pH, nitric oxide and nitrite (or free nitrous acid HNO_2) concentration (Schulthess et al., 1995; Otte et al., 1996; Zhou et al., 2008; Richardson et al., 2009). The lack of biodegradable organic carbon leading to incomplete denitrification is also an important factor governing N_2O production (Hanaki et al., 1992; Park et al., 2000). Increased N_2O emissions at low C to N ratio can be caused by the indirect effect of NO_2^-/NO accumulation (Hanaki et al., 1992; Schulthess et al., 1995; Itokawa et al., 2001), and/or related to increased competition for electrons distribution among the different steps of denitrification (Pan et al., 2013a, 2013b). Although heterotrophic denitrification is considered as a source of N_2O production in wastewater treatment plants (WWTPs), it has also been identified as playing an important role to reduce the emission of N_2O produced by ammonia oxidizing bacteria (Guo, 2014; Guo and Vanrolleghem, 2014).

Until now, research focused on monitoring N_2O emissions on activated sludge WWTPs and little is known about emissions from biofilm processes. Among the available technologies, biofiltration has been widely deployed in urban wastewater treatment plants. It is the most applied technology in the Parisian area and the second one in France after activated sludge (http://assainissement.developpement-durable.gouv.fr/services.php). It combines both physical and biological treatment processes using immersed filter media. The latter acts both as a filter and as a support for the biofilm development. The benefits of this technology lie in its compactness (small foot print) and intensiveness (short residence time) (Rocher et al., 2012).

The present study aims at reporting and analyzing nitrous oxide emissions from a full-scale biofilter treatment plant located in the Parisian area (France). The denitrifying biofilters monitored were operated downstream from the biological treatment (post-denitrification). Dynamic N_2O emissions were confronted to process operating conditions in order to identify possible key parameters influencing N_2O production and consumption during denitrification.

2. Material and methods

2.1. Wastewater treatment plants description

The measurements were performed at Seine Aval WWTP, located in the Parisian area (France). The plant was designed to receive a nominal flow of 1,700,000 m^3/d (around 20 m^3/s), i.e. about 80% of the Parisian wastewater flow corresponding to about 5 million population equivalents. Wastewater is pre-treated (screening, grit removal) and passes through primary settling tanks. The water from the primary settlers enters a high loaded conventional activated sludge treatment (aerated biological reactors combined with secondary settling tanks) designed for organic carbon removal. The outlet of the activated sludge system enters a ballasted flocculation unit to remove suspended solids and phosphorus. Finally, the treated wastewater containing a low total suspended solid concentration and controlled phosphorus concentration is totally nitrified and denitrified through biofilters: 84 Biostyr® filters for nitrification followed by 18 Biostyr® and 12 Biofor® filters for post-denitrification, using methanol as an external carbon source. Since the main part of the nitrate load is treated in the Biostyr® filters, it was decided to monitor this technology.

Each Biostyr® unit has a surface area of 173 m^2 and a total volume of 605 m^3. Microbial support material, composed by 3 mm diameter polystyrene spheres, is distributed on the total biofiltre surface area over a maximum height of 3.5 m. Anoxic conditions are maintained in the biofiltres in order to obtain denitrification. However air is punctually injected during the washing procedures set up to unclog the filter media. Moreover, addition of methanol as an exogenous carbon source is performed to maintain an influent BOD to NO_3-N ratio of 3.5.

2.2. N_2O sampling and monitoring

Two measurement campaigns were performed to evaluate N_2O emissions from the denitrifying biofilters.

The first monitoring campaign (summer campaign) was conducted in September 2014 and aimed at studying a single denitrifying biofilter in order to identify the key operational conditions influencing N_2O emissions. The biofilter was monitored during seven days, corresponding to >1000 time the hydraulic retention time (HRT), and both gaseous and liquid N_2O emissions were quantified.

A second monitoring campaign (winter campaign) was performed in late January - early February 2015. Based on the summer campaign results, indicating that gaseous N_2O fluxes in denitrification were negligible in comparison to liquid emissions–see Section 3.2–, it was decided to monitor only the liquid phase. This was supported by the fact that, at low temperature, i) the higher solubility of N_2O and ii) the lower liquid to gas mass transfer would induce a higher ability of N_2O to stay in the liquid phase. The winter campaign, aimed at quantifying N_2O emissions from the whole Biostyr® denitrifying biofilters during two consecutive weeks (period corresponding to >2000 times the HRT).

The locations at which gaseous and/or liquid N_2O emissions were monitored as well as probe locations are displayed in Fig. 1.

Dissolved N_2O concentration was measured on-line with N_2O microsensors (Unisense A/S, Denmark). A daily two-point calibration was systematically performed to ensure measurement accuracy below 10%. Fresh distilled water was used for the zero point calibration whereas a fresh N_2O concentration solution was prepared for the second calibration point. This solution was obtained by bubbling a 500 ppmv standard gas in fresh distilled water. The resulting dissolved N_2O concentration was calculated according to Henry's law constant at the tested temperature.

During the summer campaign, sensors located in the biofilter inlet were placed downstream of the methanol injection point (see Fig. 1a). Despite the daily cleaning procedure, the excessive development of a biofilm certainly due to the high availability of biodegradable COD caused the failure of the dissolved N_2O sensor after 3 days of measurement. To avoid this problem during the winter campaign, the sensors and the sampler were placed upstream of the methanol injection point (see Fig. 1b).

Gaseous samples were collected using a wooden floating chamber located in the middle of the biofilter (surface area of 1.6 m^2). The gas flow was measured using a precision mass flowmeter (Brooks Instrument®, SLA 5860S, USA) with a measurement range of 50 to 500 NL/h and a detection limit of 10 NL/h. During the summer campaign, the flow was systematically measured at a value around 50 NL/h during the biofilter filtration periods. The off-gas was directed to an Infra-red analyser (AP2E, ProCeas®, France), to measure on-line the N_2O concentration. The analyser has a measurement range of 0 to 55 ppmv and a detection limit of 50 ppbv. Calibration was performed once a week using N_2O gas standards. As N_2O concentration in the exhausted gas was higher than the upper analyser's measurement limit, dilution of the gas (with pure N_2 gas) was performed using two precision flow controllers (Bronkhorst, ElFlow®, The Netherland). The dilution factor was checked every day using standards gas bottles.

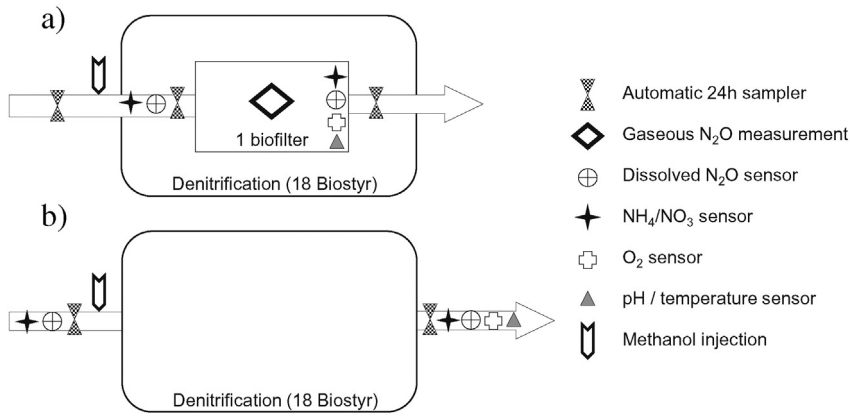

Fig. 1. Sampling strategy and sensors location for a) summer and b) winter campaign.

2.3. Wastewater sampling and analyses

In order to characterise the biofilter operating conditions and performances, a combination of on-line sensors, grab samples and composite samples was used (Fig. 1). The following on-line measurements were performed: DO (YSI, 58, USA), pH/temperature (WTW, 197I, USA), NO_3^- (WTW, Varion, USA). Grab samples were regularly collected every day to both check sensor signal (especially for NO_3^-) and measure NO_2^- and BOD concentrations. Probes cleaning were performed every morning.

Refrigerated auto-samplers were used to collect inlet and outlet samples during working days. During the summer campaign an additional auto-sampler was located upstream of the methanol injection point. During weekends, the inlet and outlet pollutant concentrations were estimated from on-line measurements and SCADA datasets for liquid flow rates. Calculated and experimental 24 h composite samples were compared and shown to be not significantly different (data not shown). Samples were analysed for NH_4^+, NO_3^-, NO_2^-, total COD, soluble COD, BOD and TSS using colorimetric Hach Lange test kits and French standard methods (AFNOR, 1994).

2.4. Fluxes and emission factor calculations

Dynamic inlet and uptake NO_3^- fluxes were calculated using continuous inlet and outlet nitrate concentrations and the inlet liquid flow as shown by Eqs. (1) and (2), respectively.

$$F_{Nin,t} = [N]_{in,t} \times Q_{in,t} \times \frac{1}{1000} \quad (1)$$

$$F_{Nup,t} = \left([N]_{in,t} - [N]_{out,t+HRT}\right) \times Q_{in,RT} \times \frac{1}{1000} \quad (2)$$

with, $F_{Nin,t}$ and $F_{Nup,t}$ the dynamic NO_3^- inlet and uptake fluxes at time t (kgN/min), $[N]_{in,t}$ the influent nitrate concentration at time t (mgN/L), $[N]_{out,t+HRT}$ the effluent nitrate concentration at time t + hydraulic retention time HRT (mgN/L), $Q_{in,t}$ the inflow at time t (m^3/min), $Q_{in,RT}$ the average inflow between t and t + HRT (m^3/min).

The dynamic gaseous N_2O flux at time t (F_{N2O}, kgN_2O-N/min) was calculated using Eq.(3).

$$F_{N_2O,t} = \left(C_{gas,t} \times Q_{air,t} \times DF\right) \times \frac{28 \times 10^{-9}}{V_m} \quad (3)$$

with C_{gas} the concentration of N_2O in the exhaust gas at time t (ppmv), $Q_{air,t}$ the gas flow coming out from the reactor at time t (L/min), DF the gas dilution factor [−] and V_m the gas molar volume determined for the temperature and pressure conditions of the measurement (L/mol).

Daily N_2O emissions (kgN_2O-N/d) were calculated as the cumulative sums of dynamics emissions during an entire day. The average N_2O emission factor (EF, %) was calculated by dividing daily liquid and/or gaseous N_2O fluxes (F_{N_2O}, kgN_2O-N/d) by the daily nitrate uptake rate (F_N, kgN/d).

Dynamic COD flux ($F_{COD,t}$, kgO_2/min) was estimated according to Eq. (4).

$$F_{COD,t} = \left[\left(Q_{meth,t} \times \rho_{meth} \times COD_{meth}\right) + \left(10^{-3} \times Q_{in} \times COD_{inf}\right)\right] \quad (4)$$

with, $Q_{meth,t}$ the methanol injection flow at time t (m^3/min), ρ_{meth} the methanol density (790 kg/m^3), COD_{meth} the COD equivalent of methanol (1.4 kgO_2/kg_{meth}), Q_{in} the daily average influent flow (m^3/min) and COD_{inf} the daily average influent COD concentration (mgO_2/L).

The dynamic BOD flux ($F_{BOD,t}$, kgO_2/min) was calculated according to Eq. (4) considering the BOD influent content (BOD_{inf}, mgO_2/L) and the BOD equivalent of methanol ($BOD_{meth} = 1\ kgO_2/kg_{meth}$).

3. Results and discussion

3.1. Influent characteristics and process performances

Influent characteristics are presented in Table 1. Process performances per active denitrifying biofilter for each measurement campaign are presented in Table 2.

The temperature during the winter campaign (14.7 ± 0.8 °C) was in average 8 °C lower than during the summer campaign (22.5 ± 0.2 °C) based on daily average values. Influent has low suspended solid (SS) content and is mainly composed of nitrate –>95% of NO_X is as nitrate. BOD and phosphorous content are high enough to allow a good denitrification activity –BOD to applied NO_X-N ratio higher than 3, and PO_4-P to applied NO_3-N ratio higher than 0.01– (deBarbadillo et al., 2006; Boltz et al., 2012; Rocher et al., 2012; Rocher et al., 2015). The influent coming from the upstream nitrification stage contained a non-negligible amount of dissolved N_2O with an average concentration of 0.3–0.4 mgN/L.

Hydraulic and nitrate loading rates per active biofilter were on average >24% higher during the winter campaign compared the summer one. As indicated in Table 2, the biofilters showed good removal performances during both monitoring periods. However, denitrification efficiency was lower during winter with an average value of 93% against 99% in summer. Besides, the average nitrite concentration in the effluent increased to a value up to 0.89 ± 0.49 mgN/L in winter whereas it was almost undetectable during summer (0.05 ± 0.02 mgN/L). These results are in accordance with those of Rocher et al. (2012) who reported denitrification rates higher than 90% in downstream denitrification biofilters

Table 1
Influent characteristics based on 24 h composite samples.

Parameter	Unit	Summer Campaign (n = 6)				Winter Campaign (n = 10)			
		Min	Max	Average	*SD*	Min	Max	Average	*SD*
Temperature	°C	22.2	22.7	**22.5**	*0.2*	13.5	15.8	**14.7**	*0.8*
pH	–	6.3	7.2	**6.7**	*0.3*	6.1	6.9	**6.7**	*0.2*
COD	mgO_2/L	168	208	**191**	*15*	131[a]	230[a]	**191**[a]	*36*[a]
BOD	mgO_2/L	111	126	**116**	*5*	73[a]	117[a]	**98**[a]	*16*[a]
TKN	mgN/L	6.3	8.7	**7.1**	*0.9*	5.6	7.5	**6.7**	*0.6*
NO_X	mgN/L	28.5	32.7	**30.3**	*1.6*	22.0	35.5	**29.3**	*4.8*
N_2O_d	mgN/L	0.1	0.3	**0.3**	*0.2*	0.3	0.6	**0.4**	*0.1*
TP	mgP/L	1.2	1.6	**1.4**	*0.1*	0.7	1.1	**0.9**	*0.2*
COD/NO_X	mgO_2/mgN	5.4	6.9	**6.3**	*0.6*	5.9	8.0	**6.5**	*0.7*
BOD/NO_X	mgO_2/mgN	3.5	4.2	**3.8**	*0.2*	2.8	4.1	**3.4**	*0.3*
PO_4/NO_X	mgP/mgN	0.021	0.026	**0.024**	*0.002*	0.007	0.027	**0.016**	*0.006*

SD, standard deviation.
[a] COD and BOD content calculated according to the daily average influent concentration and the dynamic methanol dosing (see Eq.(4)).

operated with similar conditions of the present study (BOD/N > 3 and applied nitrate load <4 $kgN/m^3/d$).

3.2. Evaluation of N_2O dynamics and emission factors

In order to evaluate N_2O liquid-gas distribution, both gaseous and liquid N_2O fluxes were monitored on a single biofilter unit during the summer campaign (data not shown). The results indicated that the gaseous flux contributed in average to <1% of the total N_2O flux (gas + liquid), meaning that >99% of nitrous oxide flux remained in the liquid phase and was discharged in the ecosystem with the effluent. This can be explained by the weak N_2O liquid-to-gas exchange due to reduced transfer at the unit surface. Therefore, N_2O emissions from denitrifying biofilters can be quantified with an accurate monitoring of the liquid phase.

Dissolved nitrous oxide fluxes as well as inlet nitrate loads are presented in Fig. 2. Daily emission factors and removal of dissolved N_2O are presented in Table 3.

During the summer campaign (Fig. 2a), the inlet nitrate load regularly dropped to zero which corresponds to periods where the feeding of the biofilter unit was stopped. The duration of these periods varied from 2 min to almost 2 h. These feeding interruptions correspond (i) to washing periods and (ii) to automatic shutdowns occurring when the influent flow reaches a given set-point below which the low upstream velocity causes a too high clogging speed of the biofilter. This phenomenon was not observed on the winter campaign data (reported in Fig. 2b) because the influent flow used to calculate the nitrate load corresponded to the total inlet flow of the denitrifying Biostyr® stage divided by the number of active biofilters. Hence, this latter does not reflect the high variability of the flow in the immediate inlet of a given biofilter unit.

During both monitoring periods, the inlet dissolved N_2O flux was, most of the time, higher than the outlet flux (see Fig. 2). Daily removal of dissolved N_2O ranged from −14% to 95% in summer (only for the first three days of monitoring) and from 26% to almost 100% in winter (see Table 3). Except for day 1 of the summer campaign (9/25 to 9/26), the daily removal of N_2O was always positive indicating a consumption of part of the inlet dissolved N_2O flux. This underlines that emission factors reported in Table 3 do not transcribe a real production of N_2O from the denitrifying biofilters but represent the remaining part of the dissolved N_2O that has not been consumed in the denitrification stage. Under usual process operating conditions of the summer campaign –BOD/NO_3-N > 3– apparent N_2O uptake rate was estimated to be on average of 35.5 ± 8.7 $gN_2O\text{-}N/m^3/d$. Adding the quantity of N_2O produced during the reduction of nitrate, the daily average N_2O reduction rate was estimated at 1363 ± 163.1 $gN_2O\text{-}N/m^3/d$ (1 mole NO_3 gives ½ mole N_2O considering no NO_2^- accumulation).

Several studies have already reported a sink of N_2O during biological nitrogen removal (Foley et al., 2010; Hu et al., 2011; Filali et al., 2013). Under optimal denitrifying conditions, N_2O reductase enzyme has a higher maximum nitrogen turnover than NO_2 and NO_3 reductases (Vonschulthess et al., 1994; Wicht, 1996). This indicates not only that N_2O from the reduction of nitrites could be completely reduced during denitrification, but also that heterotrophic denitrification can reduce part of N_2O produced by ammonia oxidizing bacteria. Those results are consistent with the studies of Guo (2014) and Guo and Vanrolleghem (2014), which reported that heterotrophic denitrification plays an important role in the reduction of N_2O produced by ammonia oxidizing bacteria in a full-scale activated sludge WWTP.

Whereas on average a consumption of N_2O in the denitrification stage was monitored, several peaks of N_2O with medium to high intensity have been regularly observed during both monitoring periods. Several peaks with high intensity were observed during the summer campaign (day 1 from 9/25 to 9/26; and then day 4 to 5 from 9/28 to 9/30). Three major peaks were observed during days 9 and 10 (from 2/3 to 2/5) of the winter campaign. Analysis of the operating conditions of the biofilter unit monitored during that campaign showed that the medium intensity N_2O production peaks happened most of the time

Table 2
Operating conditions and process performances per active biofilter.

		Operating conditions per active biofilter				Number of active biofilters
		Hydraulic loading rate $m^3/m^2/d$	Nitrate loading rate $kgN/m^3/d$	Nitrate removal rate $kgN/m^3/d$	Denitrification efficiency %	
Summer campaign (n = 7)	Min	59	1.71	1.70	98.6	11.0
	Max	85	2.70	2.68	99.3	18.0
	Average	**75**	**2.26**	**2.24**	**98.9**	**16.2**
	SD	*10*	*0.37*	*0.37*	*0.2*	–
Winter campaign (n = 14)	Min	72	1.83	1.78	85.7	11.0
	Max	118	3.07	2.98	99.3	18.0
	Average	**97**	**2.76**	**2.66**	**93.1**	**17.2**
	SD	*15*	*0.33*	*0.33*	*4.2*	–

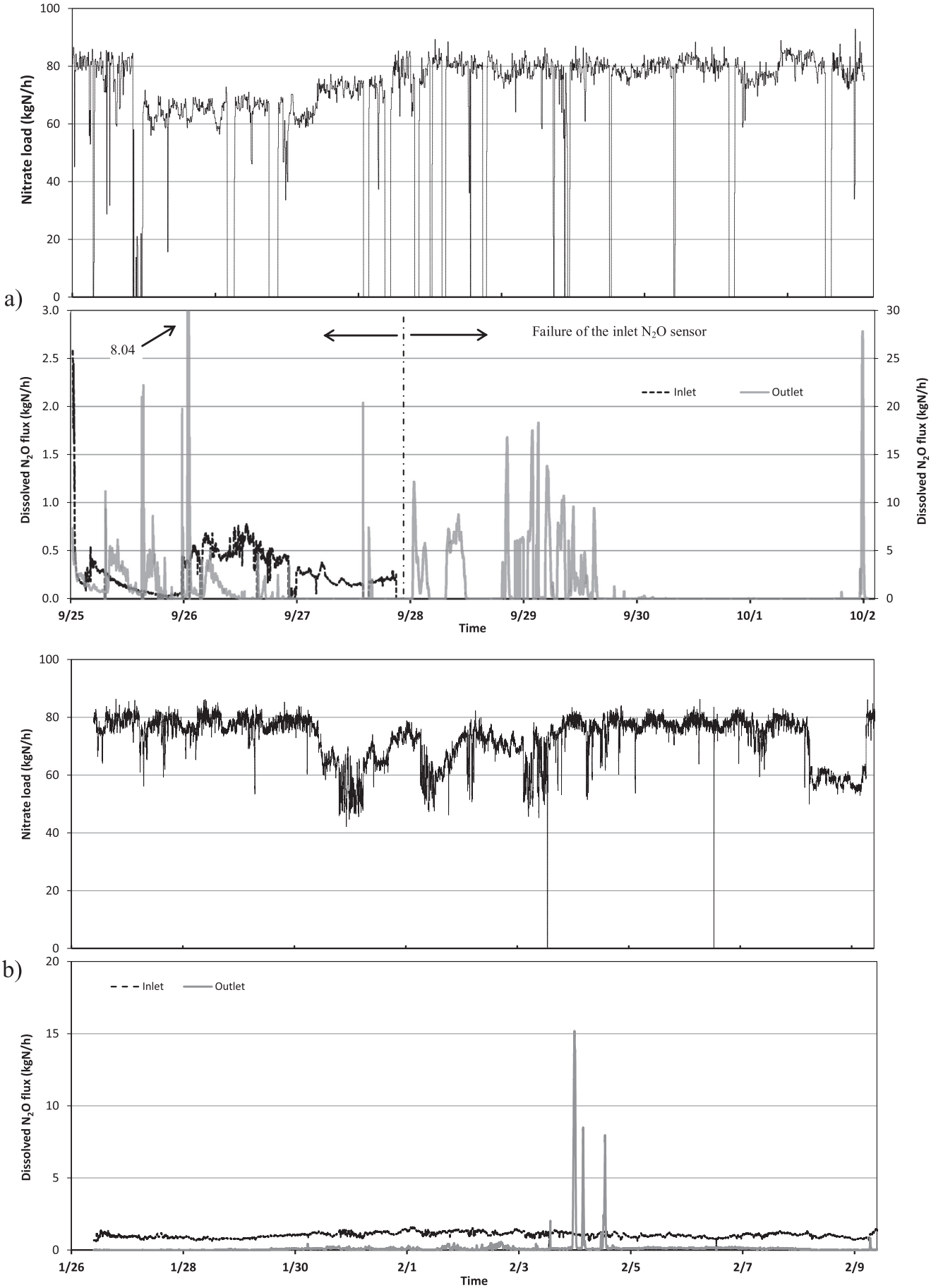

Fig. 2. Influent NO_3 load and dissolved N_2O flux per active biofilter for a) summer campaign, left scale from 9/25 to 9/28, right scale from 9/28 and b) winter campaign.

Table 3
Daily emission factor and removal of dissolved N_2O.

Day	Summer Campaign		Winter Campaign	
	Liquid EF N_2O-N/NO_3-N_{uptake} (%)	Removal N_2O %	Liquid EF N_2O-N/NO_3-N_{uptake} (%)	Removal N_2O %
1	0.305	−13.6	0.002	99.9
2	0.241	64.0	0.003	99.7
3	0.019	95.4	0.012	98.9
4	4.252		0.094	93.2
5	4.121		0.187	90.2
6	0.011		0.116	93.9
7	0.008		0.152	92.6
8			0.241	86.9
9			1.216	25.9
10			0.505	68.7
11			0.194	86.2
12			0.188	85.0
13			0.114	92.5
14			0.052	97.4
Average	**1.279**	**48.6**	**0.220**	**86.5**
SD	*1.990*	*56.1*	*0.314*	*19.2*
Average*			**0.11**	**93.0**
*SD**			*0.08*	*5.3*

*Values without methanol failure episodes of the winter campaign (days 9 and 10).

during and/or after biofilter washing procedure. Some of N_2O peaks with high intensity observed during both campaigns were linked to process operating conditions and this part will be discussed thereafter (Section 3.3).

The daily N_2O emission factor detailed on Table 3 were highly variable and were estimated to be on average of 1.28 ± 1.99% NO_3-N_{uptake} and 0.22 ± 0.31% NO_3-N_{uptake} during summer and winter campaigns, respectively. It is to be noted that as both emission factors have not been measured in the same way (single biofilter in the summer campaign against whole denitrification unit during the winter campaign) they cannot be directly compared. For example, an increase of the outlet dissolved N_2O concentration that would have happened on a single biofilter during the winter campaign would not necessarily have been detected in the total outlet due to the dilution with the effluent from the other biofilters. Emission factors of the present study are comparable with those estimated on a pilote-scale denitrifying biofilter (Tallec et al., 2006). Authors reported emission factors ranging from 0.2% to 1.2% depending on the influent BOD/N ratio. Wunderlin et al. (2012) performed batch experiments on activated sludge for wastewater treatment. Reported emissions factors were of 0.2% in pure anoxic conditions and much higher (0.8% to 19%) in the presence of oxygen during nitrate reduction.

The IPCC, 2006 Guidelines for National Greenhouse Inventories considered that 0.5% of wastewater effluent total nitrogen discharged into rivers and/or estuaries can be emitted as N_2O. In order to compare the present results with the IPCC calculation, the hypothesis that the totality of the measured outlet dissolved N_2O fluxes will be transferred into the gas phase in rivers (assuming no reduction of N_2O via denitrification in rivers) was made. No transformation of total nitrogen to N_2O in rivers was considered. In these conditions, the results of the present study indicate that N_2O emissions are 18 (due to the high N_2O emissions during days 4 and 5) and 2 times higher than those estimated with the default IPCC factor for indirect emissions for summer and winter campaigns, respectively. These evaluations could be further increased if considering a downstream transformation of total nitrogen into N_2O.

3.3. Impact of operating parameters on N_2O emissions

A cross analysis of N_2O peaks and operating conditions of the biofilters indicated that most of N_2O production was related to the presence of oxygen and to the variation of the influent BOD to N ratio.

3.3.1. Impact of dissolved oxygen

The biofilter washing procedure is automatically triggered when material clogging, reaches a given set-point value (measured with pressure loss in the filtration media). Washing consists in alternate injections of air and water in the filter unit, which removes the excess biomass. The washing procedure was carried out every 20–30 h and its duration was around 30 min. Fig. 3 presents the typical evolution of N_2O emissions with dissolved oxygen concentration during and after a given washing period (day 2 of summer campaign corresponding to the period from 9/26 to 09/27).

Whereas oxygen concentration in the effluent of the denitrifying biofilter remained close to zero during filtration period, it increased above 1 mg/L during the washing period (from 3:00 AM to 3:25 AM) and decreased rapidly after the recovery of the biofilter activity. The increase of oxygen concentration was concomitant with both the increase of gaseous N_2O emission rate and effluent dissolved N_2O concentration. The increase of N_2O in both phases indicates a production of dissolved N_2O and its subsequent transfer into the gas phase. Unlike oxygen, N_2O emissions did not decrease immediately after the recovery of the biofilter activity and it took almost 4 h to reach the level obtained before the washing period.

During denitrification, dissolved oxygen (DO) is known for triggering N_2O emissions as oxygen inhibits both synthesis and activity of denitrification enzymes (Otte et al., 1996). Bonin et al. (1992) showed that N_2OR was the denitrification enzyme the most sensitive to oxygen in *Pseudomonas nautica*. In lab-scale experiments, a positive correlation between N_2O emissions and low DO concentrations (under 0.7 mg/L)

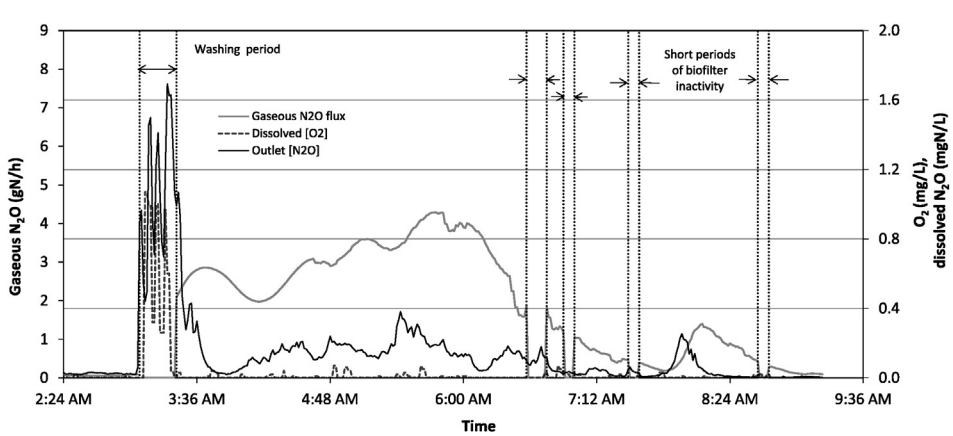

Fig. 3. Effect of dissolved oxygen on N_2O emission during and after a washing period (day 2 of the summer campaign). *The increase of gaseous N_2O flux during the washing period was not represented in this figure to allow better visibility of changes in dissolved oxygen and N_2O concentration.*

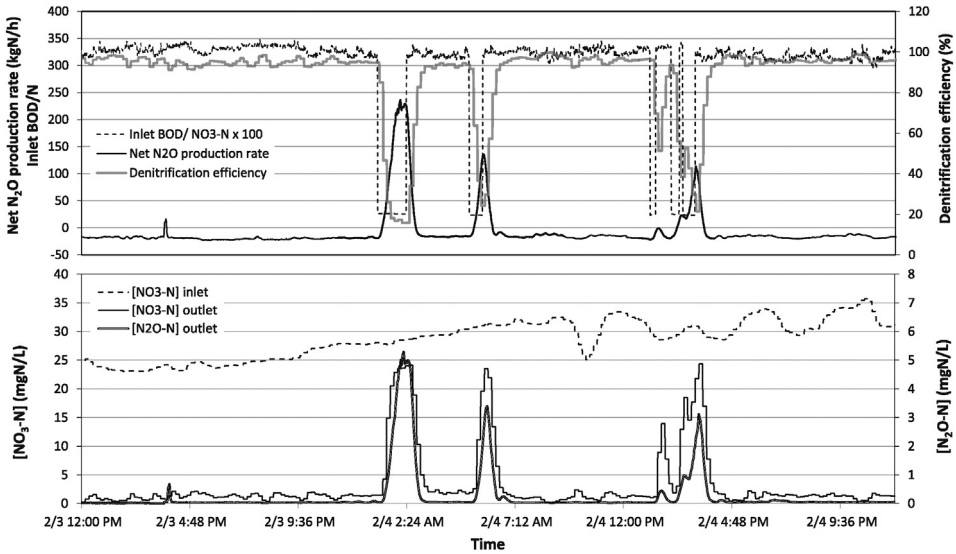

Fig. 4. Impact of influent BOD/N ratio on denitrification efficiency and N_2O emissions (day 9 and part of day 10 of the winter campaign).

was established by Gong et al. (2012). Authors reported nitrite accumulation with increased DO concentration and linked N_2O emissions to a possible inhibition of N_2O reductase by nitrite. Wunderlin et al. (2012) reported N_2O emissions factors between 4 and 90 times higher in the presence of low DO concentrations (0.1–0.2 mg/L) compared to pure anoxic conditions. However, these experiments were conducted in DO-stabilized conditions and did not focus on the study of the effect of DO changing conditions on N_2O emissions.

Otte et al. (1996) studied the influence of anaerobic-aerobic transitions on enzymes of *Alcaligenes faecalis*. They showed that oxygen application after a non-aerated period induced a rapid N_2O emission due to the near instantaneous N_2O reductase (N_2OR) inhibition whereas nitrite reductase and nitrate reductase maintained their activities, involving N_2O accumulation. In other experiments, changing from aerobic to anaerobic conditions, the same authors found that N_2OR responded fast to aerobic-anaerobic transient conditions but reached its maximum activity within 7 h. Authors reported also that after a few cycles with repetitively changing aerobic-anaerobic conditions, both N_2O emissions and latency time decreased indicating bacterial adaptation to the operating conditions of the reactor. The present experimental results corroborate the observations reported in the literature. The rapid increase in N_2O emission after air supply could be explained by the inhibitory effect of DO on N_2OR as described by Otte et al. (1996). The possible influence of nitrite cannot be discussed as not enough measurements of nitrite concentration were available during washing periods. The latency that could correspond to the time necessary for N_2OR to reach its maximum activity was often observed but its duration was variable between each washing period (from 1 to several hours).

Overall, N_2O emissions occurring during the 30 min of the washing periods represented <1% of the average daily emissions. However, as discussed above the impact of the presence of oxygen on N_2O emissions extends well beyond washing periods but remains low in comparison to the impact of carbon availability described hereafter.

3.3.2. Impact of BOD/N ratio

Impact of carbon availability on N_2O emissions was evaluated in comparison to influent BOD/N dynamics during the monitoring periods. Fig. 4 presents the evolution of the denitrification efficiency and N_2O emissions with influent BOD/N ratio during days 9 and 10 of the winter campaign.

Inlet BOD/N-NO_X was on average of 3.8 ± 0.2 kgO_2/kgN and 3.4 ± 0.3 kgO_2/kgN during summer and winter campaigns, respectively. However, three episodes of methanol injection failure occurred at night during days 9 and 10 of the winter campaign (from 2/3 to 2/5). During these episodes methanol was not added to the influent and BOD/N ratio rapidly dropped under a value of 0.5 kgO_2/kgN (see Fig. 4). The first and second failure episodes lasted respectively 75 min and 35 min whereas the third one actually consisted of three consecutive failures lasting 13, 21 and 33 min on a time window of 2 h. The decrease of BOD/N during these episodes was rapidly followed by a strong collapse of denitrification efficiency that decreased to 50%–15% depending on the duration of the methanol injection failure. Whereas the net N_2O production rate was negative when BOD/N ratio was higher than 3 (average value of −16.5 kgN_2O-N/h), it increased up to 236, 135 and 114 kgN_2O-N/h during the three methanol injection failures.

The collapse of denitrification efficiency during episodes of methanol injection failure is explained by the fact that the effluent coming from the upstream activated sludge reactor contains a low content of carbon (COD of 60 mg/L on average) which is the electron donor of the reaction. Using methanol as an external carbon source, the amount of COD required to denitrify 1 g of NO_3-N would be of 4.47[2] – corresponding to a BOD/N ratio of 3.2. Recently, Rocher et al. (2015) investigated the impact of the carbon dosage on nitrite accumulation in full-scale post-denitrification biofilters and found that the BOD/N ratio allowing a complete denitrification with minimal nitrite accumulation (<1 mgN/L) ranges from 3.0 to 3.2.

A lack of carbon has been widely reported as a promoting factor of N_2O emissions in activated sludge WWTPs (Kampschreur et al., 2009; Law et al., 2012). Itokawa et al. (2001) reported that 20 to 30% of inlet nitrogen was converted to N_2O at low C/N ratio (i.e 3.5 and 2.4) whereas N_2O conversion lower than 1% was achieved at a COD/N ratio of 5. Kishida et al. (2004) reported that N_2O emission in the BOD/TN ratio of 2.6 was 10 times greater than that in the BOD/TN ratio of 4.5. Results of Hanaki et al. (1992) indicated increased N_2O emissions at lower COD/N ratios of 1.5 to 2.5. Increased N_2O emissions at low carbon to nitrogen ratio was generally explained by the indirect effect of nitrite or nitric oxide accumulation on N_2O reductase (Hanaki et al., 1992; Schulthess et al., 1995; Oh and Silverstein, 1999). However, recent studies suggest

[2] The amount of substrate COD used to remove 1 g nitrate can be calculated as follow:

$$\frac{COD}{NO_3-N} = \frac{2.86}{1-Y}$$ (Tchobanoglous et al., 2003).

With: The 2.86 conversion factor is COD to equivalent nitrate as N, Y is the heterotrophic anoxic yield on methanol (=0.36 g COD/gCOD (Boltz et al., 2009)).

that the intensity of electron competition among the different steps of denitrification rather than C/N ratio was the main factor governing N_2O accumulation during denitrification (Pan et al., 2013a, 2013b). Results of these studies indicated that electron competition occurred under not only carbon limiting but also carbon abundant conditions. However, with electron supply being the limiting step, nitrite reductase had a higher ability to compete for electrons, in comparison with nitrate and N_2O reductase, consequently leading to N_2O accumulation.

In the present study, a rapid increase of the net N_2O production rate was observed as soon as the BOD/N ratio dropped under a value of 3; and its intensity increased with the duration of the carbon limiting conditions. During the first episode of methanol injection failure (the longest one) the net N_2O production rate attained 17% of inlet nitrate load which is consistent with the results of Itokawa et al. (2001). Even though episodes of methanol injection failure lasted <2 h, they greatly degraded the apparent daily removal of dissolved N_2O. This later decreased to 26% and 69% at day 9 and 10 whereas it was on average of 93% for the other days. Results of the present study indicate that carbon availability not only influences denitrification rate but has also a major impact on N_2O emissions in post-denitrifying biofilters.

Whether N_2O production was related to electron competition or to the accumulation of inhibiting products such as nitrite and nitric oxide remains unclear. Rocher et al. (2015) indicated that for important carbon underdosing (BOD/N < 2), biofilters denitrification effluent contains nitrate but very few nitrite. Accumulation of nitrite was observed for moderate underdosing conditions (BOD/N of 2.3 to 2.4). However, these concentrations were measured in the effluent and nitrite accumulation in the filter media could have been more important especially in conditions of methanol underdosing. Since methanol dosing failures happened at night, nitrite measurement was not performed and measurement of gaseous NO emissions not included in our protocol. Regardless the mechanism of N_2O production, results of the present study indicates that the BOD/N ratio should be maintained above a value of 3 in order to maximize the reduction of dissolve N_2O coming from the upstream nitrification stage and minimize the accumulation of N_2O during the reduction of nitrate.

3.4. General discussion

The analysis of N_2O emissions in the denitrification stage indicated that the presence of oxygen and conditions of methanol underdosing were responsible for most of N_2O production. However some N_2O peaks with high intensity observed during the summer campaign and measured in both the gas and liquid phases (day 1 from 9/25 to 9/26 and day 4 to 5 from 9/28 to 9/30 on Fig. 2) have not been fully explained. Indeed, during these days most of operating conditions of the biofilters were similar to the ones occurring when low N_2O emission is observed (pH, temperature, influent characteristics, methanol dosing). The only parameter that changed was nitrate load that decreased on days 2 and 3 and gradually increased on day 4 to reach its initial value observed at the beginning of the campaign. One can suppose that such an increase in the nitrate load can induce nitrite accumulation favoring N_2O emissions but the average concentration of nitrite in the effluent remained very low (0.08 mgN/L). Random grab samples performed during days 1, 4 and 5 indicated that nitrite concentration was low and remained in the same range as previous days.

The effect of carbon addition and oxygen content on N_2O emissions has been partially linked to nitrite concentration as the literature highly reports this parameter as favoring N_2O emissions. This assumption has not been categorically verified as no sufficient data were available in terms of nitrite concentrations during these N_2O emission periods. However, measurement of nitrite concentration clearly show that in comparison with activated sludge, the nitrite threshold concentrations reported are possibly different in biofilters processes. During usual denitrification operation (BOD/N > 3, O_2 < 0.1 mg/L), specific measurements made at different heights in the filtration media during the winter campaign (data not shown, similar to those observed by Rocher et al. (2015)) indicated that nitrite concentration could reach values as high as 5 mgN/L. In these conditions no N_2O emissions was measured and a consumption of N_2O was even observed. As N_2O was measured in the biofilter outlet, the effective N_2O dynamics within the media could not be observed. Additional measurements to investigate the effect of nitrite on N_2O dynamics in denitrifying biofilter are required. In particular, the possible acclimatisation of the organisms involved in the N_2O cycle in response to the presence of permanent high nitrite concentrations and the effect of changing nitrate load on N_2O emissions must be clarified.

4. Conclusions

N_2O emissions from a full-scale denitrifying biofilter plant were monitored over two periods with the objective of identifying possible key parameters influencing N_2O production and consumption during denitrification.

Comparison of gaseous and liquid N_2O fluxes indicated that >99% of the flux remained in the liquid phase and was discharged to the receiving body with the effluent. From a metrological point of view, this result suggests that in denitrifying reactors: (i) emissions can be quantified with an accurate monitoring of the liquid phase and (ii) the currently used methods to estimate the N_2O emissions from WWTPs should take into account effluent dissolved N_2O fluxes. Moreover, denitrification was found to be able to reduce on average 86% of the dissolved N_2O flux coming from the upstream nitrification stage. In the absence of methanol injection failure, N_2O breakdown was on average of 93%. Analysis of the conditions of N_2O appearance indicated that influent BOD to N ratio should be maintained above a value of 3 in order to minimize N_2O emission.

Several N_2O production events monitored during the first campaign could not be fully understood based on the analyses performed on the inlet and outlet of the reactor. Further investigation of the dynamic of carbon and nitrogen oxides within the filtration media is required. Additional full-scale measurements of N_2O emissions from biofilters WWTPs are required in order to better assess the variability of emissions and to specify N_2O emission factor of this technology.

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References

AFNOR, 1994. Qualité de l'eau Recueil de normes Afnor Editions.

Boltz, J.P., Johnson, B.R., Daigger, G.T., Sandino, J., Elenter, D., 2009. Modeling integrated fixed-film activated sludge and moving-bed biofilm reactor systems II: evaluation. Water Environ. Res. **81** (6), 576–586.

Boltz, J.P., Morgenroth, E., Daigger, G.T., deBarbadillo, C., Murthy, S., Sørensen, K.H., Stinson, B., 2012. Method to identify potential phosphorus rate-limiting conditions in post-denitrification biofilm reactors within systems designed for simultaneous low-level effluent nitrogen and phosphorus concentrations. Water Res. **46** (19), 6228–6238.

Bonin, P., Gilewicz, M., Bertrand, J., 1992. Effects of oxygen on *Pseudomonas nautica* growth on n-alkane with or without nitrate. Arch. Microbiol. **157** (6), 538–545.

deBarbadillo, C., Rectanus, R., Canham, R., Schauer, P., 2006. Tertiary denitrification and very low phosphorus limits: a practical look at phosphorus limitations on denitrification filters. Proceedings of the Water Environment Federation, WEFTEC.

Filali, A., Fayolle, Y., Peu, P., Philippe, L., Nauleau, F., Gillot, S., 2013. Aeration control in a full-scale activated sludge wastewater treatment plant: impact on performances, energy consumption and N2O emission. 11th IWA Conference on Instrumentation Control and Automation ICA, Narbonne, France.

Foley, J., de Haas, D., Yuan, Z., Lant, P., 2010. Nitrous oxide generation in full-scale biological nutrient removal wastewater treatment plants. Water Res. **44** (3), 831–844.

Gong, Y.-K., Peng, Y.-Z., Yang, Q., Wu, W.-M., Wang, S.-Y., 2012. Formation of nitrous oxide in a gradient of oxygenation and nitrogen loading rate during denitrification of nitrite and nitrate. J. Hazard. Mater. **227–228**, 453–460.

Guo, L., 2014. Greenhouse Gas Emissions from and Storm Impacts on Wastewater Treatment Plants: Process Modelling and Control (PhD) - Université Laval.

Guo, L., Vanrolleghem, P., 2014. Calibration and validation of an activated sludge model for greenhouse gases no. 1 (ASMG1): prediction of temperature-dependent N2O emission dynamics. Bioprocess Biosyst. Eng. **37** (2), 151–163.

Hanaki, K., Hong, Z., Matsuo, T., 1992. Production of nitrous-oxide gas during denitrification of waste-water. Water Sci. Technol. **26** (5–6), 1027–1036.

Hu, Z., Zhang, J., Xie, H., Li, S., Zhang, T., Wang, J., 2011. Identifying sources of nitrous oxide emission in anoxic/aerobic sequencing batch reactors (A/O SBRs) acclimated in different aeration rates. Enzym. Microb. Technol. **49** (2), 237–245.

IPCC, 2006. Vol. 1 – general guidance and reporting. Prepared by the National Greenhouse Gas Inventories Programme. In: Eggleston, H.S., Buendia, L., Miwa, K., Ngara, T., Tanabe, K. (Eds.), 2006 IPCC Guidelines for National Greenhouse Gas Inventories. IGES, Japan.

IPCC, TF, Stocker, Qin, D., G-K, Plattner, Tinor, M., SK, Allen, Boschung, J., Nauels, A., Xia, Y., Bex, V., PM, Midgley, 2013. Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergouvernmental Panel on Climate Change. Cambridge University Press; 2013, Cambridge, United Kingdom and New York, NY, USA (1535 pp.).

Itokawa, H., Hanaki, K., Matsuo, T., 2001. Nitrous oxide production in high-loading biological nitrogen removal process under low cod/n ratio condition. Water Res. **35** (3), 657–664.

Kampschreur, M.J., Temmink, H., Kleerebezem, R., Jetten, M.S.M., van Loosdrecht, M.C.M., 2009. Nitrous oxide emission during wastewater treatment. Water Res. **43** (17), 4093–4103.

Kishida, N., Kim, J.H., Kimochi, Y., Nishimura, O., Sasaki, H., Sudo, R., 2004. Effect of C/N ratio on nitrous oxide emission from swine wastewater treatment process. Water Sci. Technol. **49** (5–6), 359–365.

Law, Y., Ye, L., Pan, Y., Yuan, Z., 2012. Nitrous oxide emissions from wastewater treatment processes. Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci. **367** (1593), 1265–1277.

Oh, J., Silverstein, J., 1999. Acetate limitation and nitrite accumulation during denitrification. J. Environ. Eng. **125** (3), 234.

Otte, S., Grobben, N.G., Robertson, L.A., Jetten, M.S., Kuenen, J.G., 1996. Nitrous oxide production by *Alcaligenes faecalis* under transient and dynamic aerobic and anaerobic conditions. Appl. Environ. Microbiol. **62** (7), 2421–2426.

Pan, Y., Ni, B.-J., Bond, P.L., Ye, L., Yuan, Z., 2013a. Electron competition among nitrogen oxides reduction during methanol-utilizing denitrification in wastewater treatment. Water Res. **47** (10), 3273–3281.

Pan, Y., Ni, B.-J., Yuan, Z., 2013b. Modeling electron competition among nitrogen oxides reduction and N2O accumulation in denitrification. Environ. Sci. Technol. **47** (19), 11083–11091.

Park, K.Y., Inamori, Y., Mizuochi, M., Ahn, K.H., 2000. Emission and control of nitrous oxide from a biological wastewater treatment system with intermittent aeration. J. Biosci. Bioeng. **90** (3), 247–252.

Ravishankara, A.R., Daniel, J.S., Portmann, R.W., 2009. Nitrous oxide (N2O): the dominant ozone-depleting substance emitted in the 21st century. Science **326** (5949), 123–125.

Richardson, D., Felgate, H., Watmough, N., Thomson, A., Baggs, E., 2009. Mitigating release of the potent greenhouse gas N2O from the nitrogen cycle – could enzymic regulation hold the key? Trends Biotechnol. **27** (7), 388–397.

Rocher, V., Paffoni, C., Goncalves, A., Guerin, S., Azimi, S., Gasperi, J., Moilleron, R., Pauss, A., 2012. Municipal wastewater treatment by biofiltration: comparisons of various treatment layouts. Part 1: assessment of carbon and nitrogen removal. Water Sci. Technol. **65** (9), 1705–1712.

Rocher, V., Laverman, A., Gasperi, J., Azimi, S., Guérin, S., Mottelet, S., Villières, T., Pauss, A., 2015. Nitrite accumulation during denitrification depends on the carbon quality and quantity in wastewater treatment with biofilters. Environ. Sci. Pollut. Res. **22** (13), 10179–10188.

Schulthess, R.V., Kühni, M., Gujer, W., 1995. Release of nitric and nitrous oxides from denitrifying activated sludge. Water Res. **29** (1), 215–226.

Tallec, G., Garnier, J., Gousailles, M., 2006. Nitrogen removal in a wastewater treatment plant through biofilters: nitrous oxide emissions during nitrification and denitrification. Bioprocess Biosyst. Eng. **29** (5–6), 323–333.

Tchobanoglous, G., Burton, F.L., Stensel, H.D., Metcalf and Eddy, 2003. Wastewater Engineering: Treatment and Reuse. McGraw-Hill.

Vonschulthess, R., Wild, D., Gujer, W., 1994. Nitric and nitrous oxides from denitrifying activated-sludge at low-oxygen concentration. Water Sci. Technol. **30** (6), 123–132.

Wicht, H., 1996. A model for predicting nitrous oxide production during denitrification in activated sludge. Water Sci. Technol. **34** (5–6), 99–106.

Wunderlin, P., Mohn, J., Joss, A., Emmenegger, L., Siegrist, H., 2012. Mechanisms of N2O production in biological wastewater treatment under nitrifying and denitrifying conditions. Water Res. **46** (4), 1027–1037.

Zhou, Y., Pijuan, M., Zeng, R.J., Yuan, Z., 2008. Free nitrous acid inhibition on nitrous oxide reduction by a denitrifying-enhanced biological phosphorus removal sludge. Environ. Sci. Technol. **42** (22), 8260–8265.