GREENHOUSE GAS FROM MOVING BED BASED INTEGRATED FIXED FILM ACTIVATED SLUDGE MEMBRANE BIOREACTORS

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Abstract. *The present paper reports the results of a nitrous oxide production investigation in a moving bed based integrated fixed film activated sludge (IFAS) membrane bioreactor (MBR) pilot plant designed in accordance with the UCT layout for biological phosphorous removal. Samples of gas and liquid were collected in order to measure the gaseous, as well as the dissolved concentration of N_2O. Furthermore, the gas flow rate from each reactor was measured and the gas flux was estimated. The results confirmed that the anoxic reactor represents the main source of nitrous oxide production. A significant production of N_2O was, however, also found in the anaerobic reactor, thus indicating a probable occurrence of the DPAOs activity.*

1. Introduction

Nitrous oxide (N_2O) is a significant greenhouse gas (GHG) due to its high global warming potential (GWP), 298 times higher than that of carbon dioxide (CO_2), and to its capability to react with stratospheric ozone causing the layer depletion (IPCC, 2007). The requirement to protect the ozone layer led the governments worldwide to issue specific regulations and guidelines focused on the reduction of anthropogenic N_2O emissions. In this context N_2O emissions from wastewater treatment has received increasing attention in recent years.

N_2O can be produced and directly emitted from wastewater treatment plants (WWTPs). Specifically, N_2O generation mainly occurs in biological nitrogen removal (BNR) via nitrification and denitrification processes as both autotrophic and heterotrophic bacteria can be responsible for N_2O production during BNR (Kampschreur et al., 2009).

It is widely accepted that N_2O can be produced by ammonia oxidizing bacteria (AOB) via two main pathways (Kampschreur et al., 2009): i. the reduction of NO_2^- as terminal electron acceptor to N_2O (AOB denitrification) (Kim et al., 2010; Yu et al., 2010; Wrange et al., 2001; Stuven et al.,1992); ii. incomplete oxidation of hydroxylamine (NH_2OH) to NO_2^- (Law et al., 2012; Chandran et al., 2011). Furthermore, N_2O may be produced

as an intermediate of the incomplete heterotrophic denitrification (Lu and Chandran, 2010).

As it is generally recognized that the anoxic heterotrophic denitrification is the dominant process compared with aerobic denitrification and autotrophic denitrification, it is still a need to asses if this rank remains also for N_2O emission (Kampschreur et al., 2009). In the last years many efforts have been spent towards the understanding of the key mechanisms involved in N_2O production and emission (Kampschreur et al., 2009). As a consequence, several parameters that might favor N_2O production/emission have been identified: low dissolved oxygen concentrations, nitrite accumulation, dynamic conditions as well as low carbon-to-nitrogen (C/N) ratio values during denitrification. Moreover, the technical literature highlights that in processes aimed at the simultaneous nitrogen and phosphorous removal (SNPR), the role of polyphosphate accumulating organisms (PAOs) in the production of N_2O cannot be disregarded (among others, Zhou et al., 2012).

Furthermore, in a hybrid biological system (IFAS-system) where suspended as well as attached biomass is in function in the same reactor, denitrification in the biofilm even under aerobic reactor conditions, could contribute significantly to N_2O-production and emission.

In the last years, the hybrid systems have been proposed for SNPR. Hybrid systems maximize the nitrification by means of high solid retention time (SRT) of the biofilm, but having the potential of operating the suspended growth phase with a relatively short SRT. Moreover, in a hybrid system, the biofilm and the suspended biomass may function differently referring to either nitrogen or phosphorus removal. This aspect can be of importance with respect to N_2O emissions from SNPR in hybrid systems. Among the hybrid systems, the joint use of membrane bioreactors (MBRs) and moving bed biofilm reactors (MBBRs) in an IFAS-system, where the secondary is replaced by a membrane module, was recently proposed and referred to as moving bed membrane bioreactor (MB-MBR) (Di Trapani et al., 2014) or an IFAS-MBR. Up to now, only few studies (Lo et al., 2010) examined the relative effects of the biofilm and the suspended sludge on N and P removal efficiencies and N_2O emission in a hybrid SNPR system. Therefore, a better understanding of the mechanisms involved in N_2O production from hybrid systems aimed at nutrient removal is the goal of this study.

Bearing in mind such considerations, the aim of the present study was to investigate the N_2O emissions from a University of Cape Town IFAS-MBR (UCT- -IFAS-MBR) pilot plant. The UCT- -IFAS-MBR pilot plant consisted of one anaerobic, one anoxic and one aerobic compartments in series, according to the UCT scheme (Ekama et al., 1983. Particularly, the reactors were provided of specific funnel shaped covers that allowed to sample and quantify the gas flux stripped in each reactor head space.

2. Materials and methods

2.1. Pilot plant lay-out

The lay-out of the UCT- IFAS-MBR pilot plant is shown in Fig.1; it was operated for 51 days.

In details, the pilot plant consisted of anaerobic (volume: 62 L), anoxic (volume: 102 L) and aerobic (volume: 211 L) compartments (Cosenza et al., 2013). The solid-liquid separation phase was achieved by means of an ultrafiltration hollow fibre membrane module (PURON®). The membrane module was located inside a dedicated aerated compartment (referred to as the MBR tank, with a 36 L volume). An oxygen depletion reactor (ODR) allowed oxygen removal in the mixed liquor recycled from the MBR tank to the anoxic tank (Q_{RAS}). The

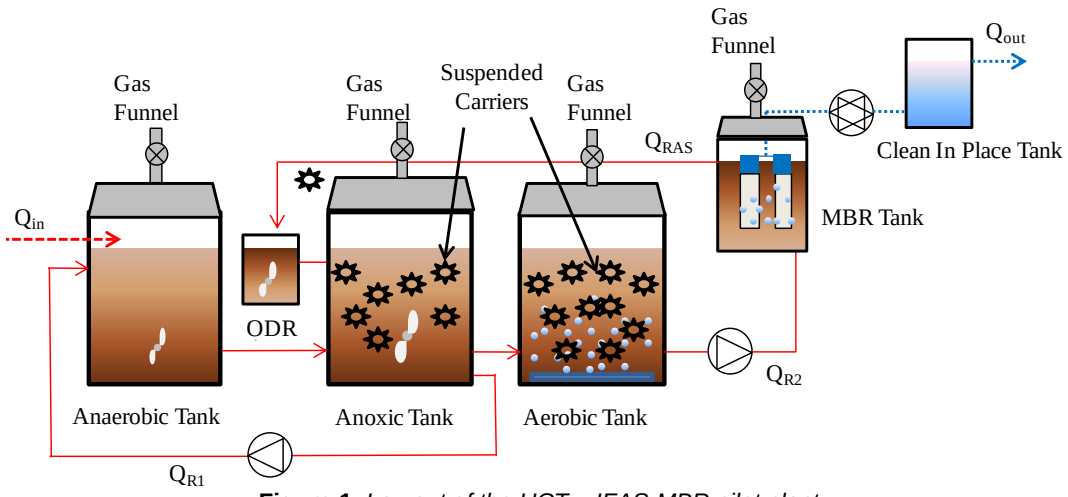

Figure 1. *Lay-out of the UCT – IFAS-MBR pilot plant*

membrane was periodically backwashed (every 9 min for a period of 1 min) by pumping a volume of permeate back through the membrane fibres from the Clean In Place (CIP) tank. The influent flow rate was set equal to 20 L h^{-1} (Q_{in}). During the pilot plant operation, a 20 L h^{-1} recycle flow (Q_{R1}) was continuously pumped from the anoxic to the aerobic tank. Furthermore, a recycle flow of 100 L h^{-1} mixed liquor (Q_{R2}) was pumped from the aerobic to the MBR tank. A net permeate flow rate of 20 L^{-1} was extracted (Q_{OUT}) through the membrane. Therefore, the recycled activated sludge (Q_{RAS}) from the MBR to the anoxic tank through the ODR tank was equal to 80 L h^{-1}. The anaerobic, anoxic, aerobic and MBR reactors were equipped with specific funnel shape covers that guaranteed gas accumulation in the headspace to perform the gas sampling.

Furthermore, the anoxic and aerobic compartments were filled with suspended carriers (Amitech s.r.l.) with a 15 and 40% filling fraction respectively, corresponding to a net surface area of 75 and 205 $m^2 m^{-3}$, respectively.

2.2. Operative conditions and experimental performances

During the experimental campaign the pilot plant was operated with no sludge withdrawal.. As the report of the present paper deals with the pilot plant gas production, the mean values of operational parameters are only briefly summarized in Table 1.

Table 1. *Mean values of operational parameters*

COD_{in} [mg L^{-1}]	TN_{in} [mg L^{-1}]	PO_4-P_{in} [mg L^{-1}]	$TSS_{anaerobic}$ [g L^{-1}]	TSS_{anoxic} [g L^{-1}]	$TSS_{aerobic}$ [g L^{-1}]	TSS_{MBR} [g L^{-1}]	VSS/TSS_{mean} -
577	60	9	1.96	3.83	3.97	4.75	0.86

On average the mean COD removal efficiency was high (>95%), the nitrification efficiency was almost complete (>95%), the denitrification efficiency was 62% and the phosphorus removal was 25%.

2.3. Gas sampling and measurements

The liquid and gaseous samples were withdrawn from the anaerobic, anoxic, aerobic and MBR tanks and analysed to determine the N_2O-N concentration. Furthermore, the N_2O-N fluxes (gN_2O-N m^{-2} h^{-1}) from all the compartments were quantified by measuring the gas flow rates, Q_{GAS} (L min^{-1}).

2.3.1. Gas flux assessment

Text Gas flow rate, expressed as Q_{GAS} was measured in accordance to Equation 1.

$$Q_{GAS} = v_{GAS} \cdot A \qquad [1]$$

where A represents the outlet section of the sampling funnel (m^2) and V_{GAS} (m s^{-1}) is the gas velocity, measured by using an hot wire anemometer.

Thus, the gas flux was assessed by applying the Equation 2.

$$F_{GAS} = \rho \cdot C \cdot \frac{Q_{GAS}}{A} \qquad [2]$$

where F_{GAS} represents the gas flux emitted from the sampled reactor (mgN_2O-N h^{-1} m^{-2}), ρ is the gas density at the recorded temperature (mol m^{-3}), C is the measured gas concentration (mg L^{-1}), Q_{GAS} is the gas flow rate (m^3 h^{-1}) and A represents the emitting surface of each sampled reactor (m^2).

2.3.2. Gas phase sampling

Gas produced due to biological activities of biomass accumulated inside the head space of each reactor funnel and was collected by withdrawing samples (9 ml) by means of commercial syringes and thus transferred into glass vials where the vacuum was previously created. Samples from the anaerobic, anoxic, aerobic and MBR reactors were collected two times per week with tree replicates for each sampling section. Gas samples were analysed with GC equipped with ECD detector to assess the Nitrous Oxide concentration.

2.3.3. Dissolved phase sampling

The measure of gas dissolved in the liquid phase was conducted on the basis of the head space gas method derived from Kimochi et al. (1998). In detail, 70 mL of supernatant (after 5 min of centrifugation at 8000 rpm) were sealed into 125 mL glass bottles. To prevent any biological reaction, 1 mL of 2N H_2SO_4 was added. After 24 h of gentle stirring, the bottles were left for 1 h without moving.

Thereafter, the gas accumulated in the headspace of the bottles was collected similarly to the gas sampling procedure. Finally, by applying Henry's Law, the dissolved gas concentration at equilibrium with the headspace gas was calculated. Samples of liquid phase were collected from the anaerobic, anoxic, aerobic, MBR and ODR reactors two times per week with tree replicates for each sampling section; furthermore liquid samples of the MBR permeate were also collected and analysed in order to assess the N_2O concentration discharged with the pilot plant effluent.

2.3.4. N_2O emission factor and mass balance

For each compartment, the evaluation of the N_2O-N emission factors, expressed as the percentage of N_2O-N emitted compared to the inlet nitrogen loading rates, was conducted by means of the following Equation 3 derived by Tsuneda et al., 2005:

$$EF = \frac{\frac{N_2O - N_{Gas}}{HRT_{HS}} + \frac{N_2O - N_{Dissolved}}{HRT}}{\frac{TN_{IN}}{HRT}} \qquad [3]$$

where EF represents the N_2O emission factor [%];N_2O-N_{Gas} is the nitrous oxide concentration in the gas phase (mg N_2O-N L^{-1}), HRT_{HS} is the hydraulic retention time of the head space of the sampled reactor, assessed by taking into account the head space volume and the gas flow rate, (h); N_2O-$N_{Dissolved}$ is the liquid phase gas concentration (mg N_2O-N L^{-1}); HRT is the hydraulic retention time of the pilot plant (h) and TN_{IN} is the total nitrogen concentration fed to the pilot plant (mgN L^{-1}).

Furthermore, in order to evaluate the gas production or consumption inside each reactor, the nitrous oxide mass balance was performed in accordance to Equation 4.

$$N_2O - N_{Dissolved,IN} - N_2O - N_{Dissolved,OUT} - N_2O - N_{Gas,OUT} = \pm N_2O - N_{p,c} \qquad [4]$$

where N_2O-$N_{Dissolved,IN}$ and N_2O-$N_{Dissolved,OUT}$ represent the influent and effluent amount of dissolved nitrous oxide respectively (mg N_2O-N h^{-1}); N_2O-$N_{Gas,OUT}$ is the effluent gaseous N_2O-N (mg N_2O-N h^{-1}) and N_2O-$N_{p,c}$ (mg N_2O-N h^{-1}) represents the amount of N_2O-N produced (in case of negative sign, therefore consider only subscript “p”) or consumed (in case of positive sign, therefore consider only subscript “c”) inside the tank.

3. Results and discussion

The recorded gas phase concentrations are shown in Figure 2. The N_2O gas concentration remained quite constant during the experimental period except for in the anoxic reactor gas concentration varied considerably as compared to the others reactors. On the 25th experimental day, the gas concentration in the anoxic reactor started to increase progressively up to the maximum value of 210 µg N_2O-N L^{-1} on the 32th experimental day. This result is likely due to an incomplete denitrification that occurred during those days, with nitrate concentration in the permeate flow ranging from 33.6 to 41 mg NO_3-N L^{-1}. This affected the gas production in the anoxic reactor, where denitrification takes place, in agreement with data in the literature where incomplete denitrification is identified as one of the causes that can lead to the N_2O production (Kampschreur et al., 2009). Several authors agree in identifying the anoxic reactor as the main N_2O producer (Otte et al., 1996; Kampschreur et al., 2009).

On average the average gas concentrations during the experimental period, measured in each reactor, were equal to 15.40 µg N_2O-N L^{-1}, 29.11 µg N_2O-N L^{-1}, 13.97 µg N_2O-N L^{-1} and 12.50 µg N_2O-N L^{-1} for anaerobic, anoxic, aerobic and MBR reactor respectively.

Based on the measured gas concentration, the flux emitted from each reactor was assessed, see Figure 3.

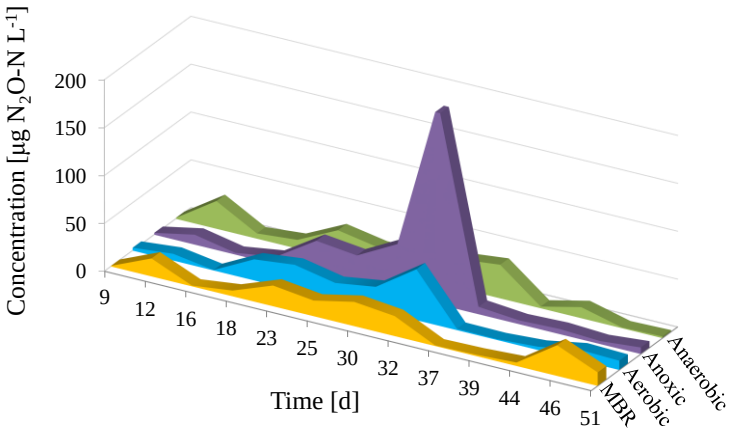

Figure 2. *Gas phase concentrations*

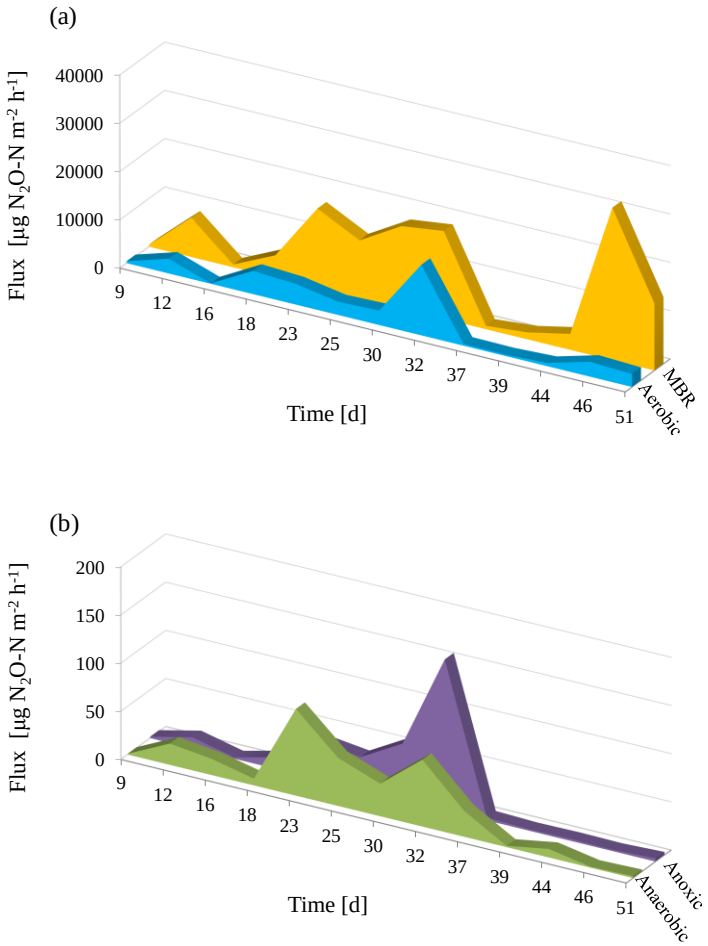

Figure 3. *N_2O-N Flux emitted from aerated reactors (a) and not aerated reactors (b)*

The results reported in Figure 3 allow analysis of the effect of the air supply to the N_2O emission as well as

the effect of the carriers. The flux measured in the aerated reactors (Figure 3a) was two orders of magnitude higher than the flux measured in the not aerated reactors (Figure 3b). The air supplied to the aerobic reactor (to guarantee the aerobic environment) and to the MBR reactor (in order to mitigate the membrane fouling) enhances the physical stripping of the gas from the liquid phase. The average fluxes measured in each reactor were found to be 25.39 μg N_2O-N m^{-2} h^{-1}, 25.01 μg N_2O-N m^{-2} h^{-1}, 3092 μg N_2O-N m^{-2} h^{-1} and 9872 μg N_2O-N m^{-2} h^{-1} for anaerobic, anoxic, aerobic and MBR reactor respectively.

The nitrous oxide flux emitted from the MBR reactor resulted on average three time higher than the flux emitted in the aerobic reactor. This result is emphasized since the scientific literature consider the nitrogen transformation process as the main source of nitrous oxide (Kampschreur et al., 2009; Law et al., 2012; Zhao et al., 2014) while in the MBR reactor, due to the low HRT (0.36 h), very little biological activity is expected to occur. The presence in the aerobic reactor of both biomasses, suspended and attached, may have enhanced the gas production both contributing to the flux emission. The reason for the huge emission from the MBR reactor is likely due to the high value of Q_{R2} (100 L h^{-1}) that is constantly pumped from aerobic to MBR reactor and thus acting as a recycle of dissolved nitrous oxide produced in the aerobic reactor. Dissolved nitrous oxide conveyed to the MBR reactor pass into the gas phase by stripping, due to the intensive aeration provided for membrane fouling mitigation, and hence contributing to the large flux emitted from MBR.

The circumstance that the physical effect of the aeration significantly affect the gas concentration is also noticeable from the dissolved gas concentrations measured in the reactors of the pilot plant (see Figure 4).

The results reported in Figure 4 provide an opposite situation for the dissolved phase as compared to the gas phase and the emitted flux of nitrous oxide. Due to the absence of the air supply, the measured concentration in the non aerated reactors (Figure 4a) resulted in an order of magnitude higher concentration than the one measured in the aerated reactors and in the permeate flow (Figure 4b). The average concentrations measured in each reactor and in the permeate flow were 100 μg N_2O-N L^{-1}, 50.30 μg N_2O-N L^{-1}, 12.25 μg N_2O-N L^{-1}, 13.12 μg N_2O-N L^{-1} and 14.63 μg N_2O-N L^{-1} for the anaerobic, anoxic, aerobic and MBR reactor and for the permeate flow respectively. The circumstance that the highest dissolved concentration were retrieved in the anaerobic reactor is likely ascribable to the low biological phosphorus removal efficiency achieved during the experimentation (25% on average). Indeed if anoxic conditions can prevail inside the anaerobic reactor, the denitrifying phosphate accumulating organisms (DPAOs) can denitrify during a denitrifying P removal process leading to a low P removal efficiency. Several studies reported that N_2O rather than N_2 was the major denitrification product when DPAOs used poly-β-hydroxyalkanoates (PHA) as a carbon source for denitrification during denitrifying P removal process (Lemaire et al., 2006; Jia et al., 2012; Wang et al., 2015). In such a context, N_2O production can be expected to be significantly influenced as the amount of anaerobic PHA synthesis is affected by influent quality variation. Hence N_2O generation during denitrifying P removal processes should also be noticed (Wang et al., 2015).

With reference to the anoxic reactor the measured N_2O concentration appears consistent with previous studies that identified the anoxic reactor as one of the major contributors to N_2O-N production (Otte et al., 1996; Kampschreur et al., 2009).

On the contrary the results reported in Figure 4b confirm that the stripping effect exerted by the air supply on the aerobic and MBR reactor is very important with respect to the flux of N_2O-N emitted. The permeate flow

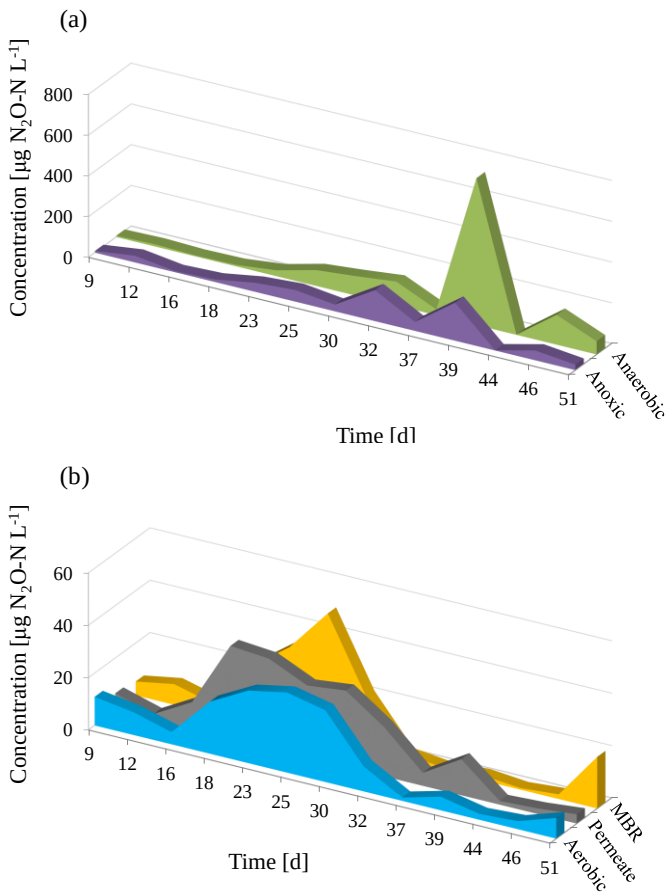

Figure 4. *Dissolved N_2O-N concentrations measured inside the anaerobic and anoxic reactors (a) and inside the aerobic and MBR reactors and in the permeate flow (b)*

concentration were comparable to dissolved concentrations measured in the other aerated reactor and thus not negligible.

In order to better quantify the influence exerted by each reactor on the N_2O emission, the emission factors have been assessed and the results are shown in Figure 5.

Results reported in Figure 5 highlight that emission factors of each compartment, expressed as a percentage of the total nitrogen load of the plant, varied moderately during the experimentation, with a maximum value close to 1%. However, Figure 5a demonstrates that the main contribution in N_2O emission derives from the aerated reactors while the contribution of the anaerobic reactor as well as of the anoxic reactor is negligible. This observation is confirmed by data reported in Figure 5b where the average contribution of each reactor is depicted. The MBR contribution represented on average 76.13% of the total emission, the aerobic reactor contributed on average with 21.38% of the total, while the sum of anaerobic and anoxic reactors contributed with a total of 2.49%. This result is consistent with the fact that the results reported in Figure 3 and 4 demonstrated that a significant fraction of N_2O produced in the reactors without air supply is further conveyed and thus stripped in the aerated reactors.

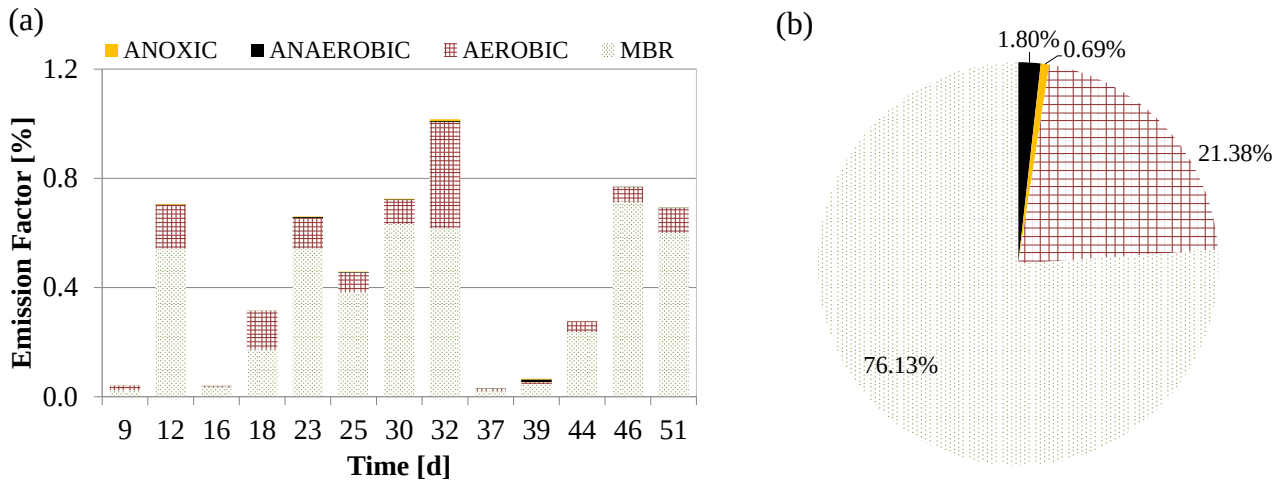

Figure 5. *N_2O-N emission factors (a) and average percentage contribution of each tank (b)*

As the denitrification is consider to be one of the dominant factor in N_2O production, the influence exerted by nitrate concentration in the nitrous oxide production is reported in Figure 6.

The data shown in Figure 6 confirm the key role of the anoxic reactor in the N_2O production. Indeed, the nitrate concentration in the anoxic reactor significantly influences the gas concentration as well as the flux (Figure 6a and b). As the nitrate concentration increase, both gas concentration and gas flux increase logarithmic. Furthermore the N_2O gaseous concentration in the anoxic reactor is strongly related to the nitrate concentration in the permeate flow (Figure 6 c).

Nitrous oxide represents an intermediate product of denitrification and thus of incomplete denitrification (Kampschreur et al., 2009). Furthermore, in the anoxic reactor the dissolved nitrate should be removed, and if biological denitrification takes place, limited N_2O production should result. The logical link between the latter observation and the presence of nitrate in the effluent is the accordance with the relation between gas concentration in the anoxic reactor and nitrate concentration in the effluent. Indeed the occurrence of high N_2O production in the anoxic reactor should represent a limitation of the denitrification phenomenon thus leading to the presence of nitrates in the effluent flow rate.

In order to investigate on the specific production/consumption of each reactor, the N_2O mass balance analysis over the reactors was carried out and shown in Figure 7.

The data presented in Figure 7 a, b, c, d and e are consistent with results previously discussed. Indeed the mass balance highlights that in the anaerobic and anoxic reactor a net N_2O production took place during the experimentation. This is in accordance with the higher N_2O dissolved concentration measured in the non aerated reactors. On the contrary, in the aerobic reactor the mass balance outlined a constant consumption of nitrous oxide (up to almost 15 mg N_2O h^{-1}), due to the high amount of gas stripped. In the MBR reactor a clear trend of the mass balance result was not noticeable. This confirms that the high nitrous oxide flux emitted from the MBR reactor was due to a physical stripping of the gas produced in other reactors of the pilot plant and conveyed in the MBR with the Q_{R2}.

However the mass balance demonstrate, with reference to N_2O production (Figure 7f), that the anaerobic and

anoxic reactors represent the main source of N_2O production (85 % of the overall production). Thus, the results of this paper indicate that the main pathways involved in the nitrous oxide production result from the heterotrophic denitrification, that normally occurs in the anoxic reactor, and the denitrifying P removal process, that is promoted by DPAOs, in the anaerobic reactor.

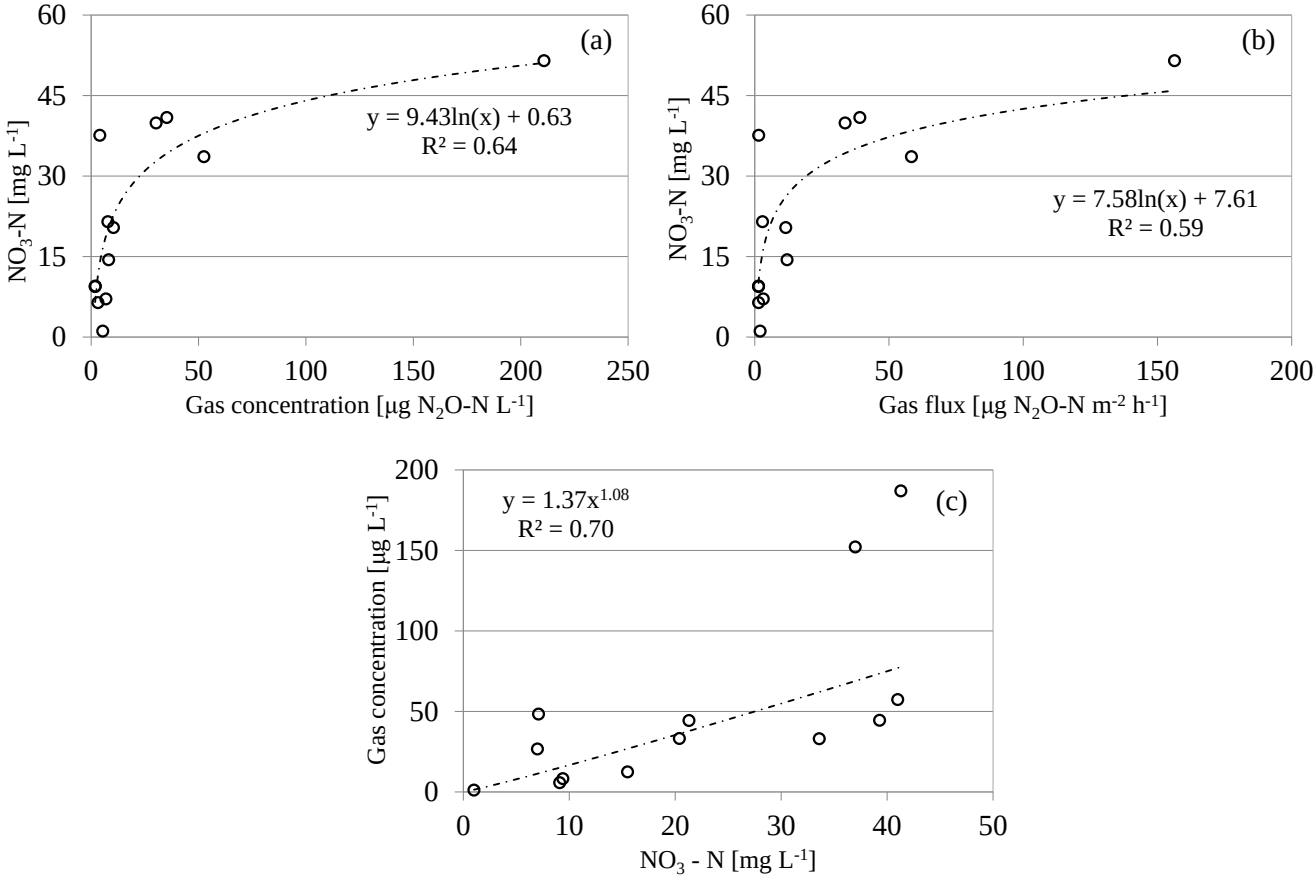

Figure 6. *Correlation betweenN_2O-N gaseous concentration and nitrate concentration in the anoxic reactor (a), nitrate concentration and N_2O-N flux in the anoxic reactor (b), N_2O-N gaseous concentration in the anoxic reactor and nitrate concentration in the permeate flow (c)*

4. Conclusions

This paper reports the results of experiments carried out in a MBBR-based IFAS MBR pilot plant operated according to the UCT design. The goal of the experiments was to investigate the nitrous oxide production.

These results indicate that the anaerobic and the anoxic reactors represent the main source of nitrous oxide production. The physical effect of aeration that caused N_2O stripping was noticed in the aerobic as well as in the MBR reactor. The N_2O flux emitted from the aerated reactors was up to two order of magnitude higher than that of the not aerated (anaerobic and anoxic) reactors. Furthermore, due to the absence of the aeration, the concentration of N_2O in the non aerated reactors was an order of magnitude higher than the concentration in the aerated reactors and in the permeate flow. The N_2O concentration measured in the permeate flow was

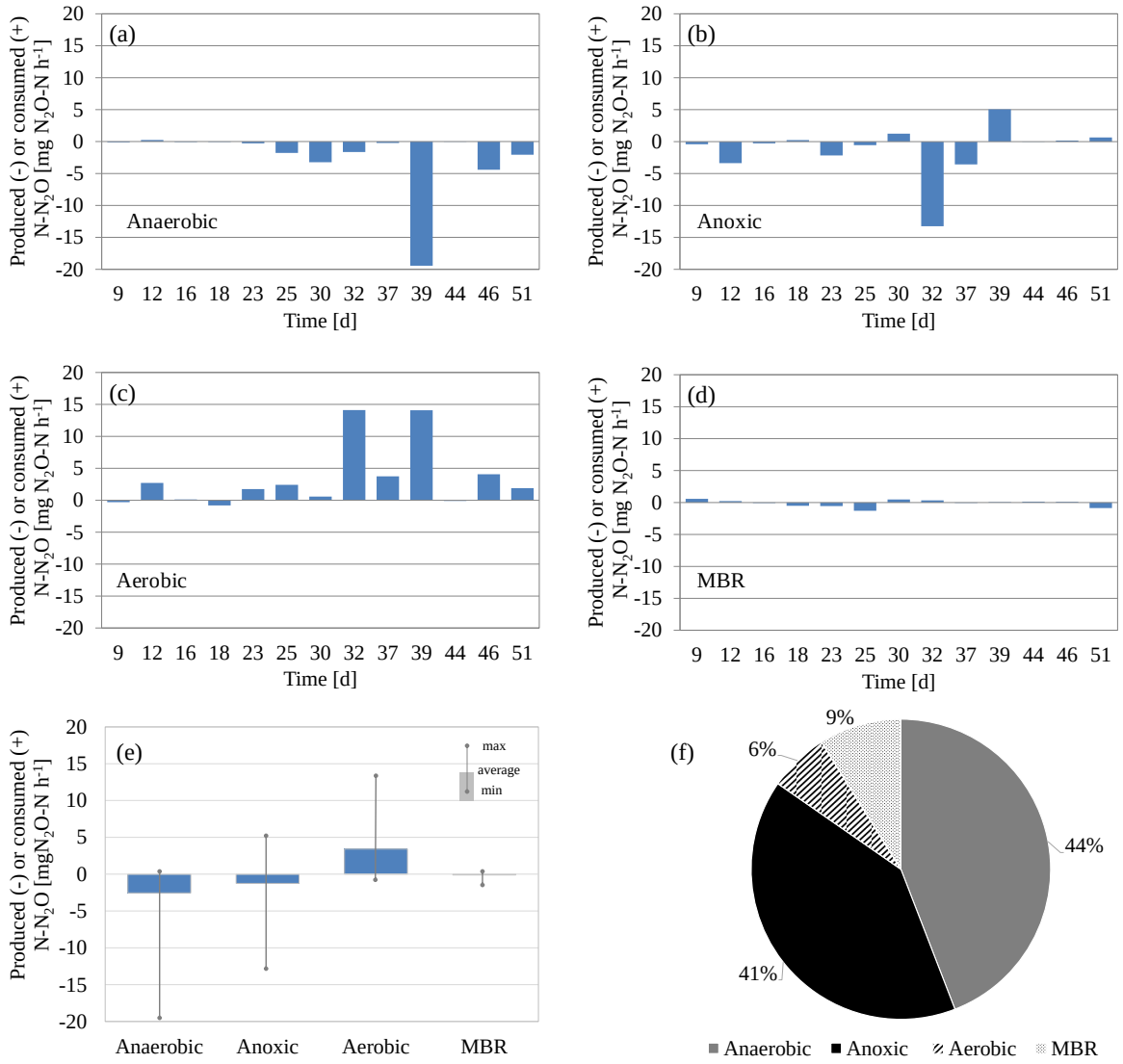

Figure 7. *N_2O-N mass balance for anaerobic (a), anoxic (b), aerobic (c) and MBR reactors (d); maximum, minimum and mean value of N_2O-N production/consumption over the reactors (e); mean percentage of N_2O-N production over the reactors (f)*

comparable to the concentrations measured in the aerobic and MBR reactors and thus not negligible. The emission factor assessment showed that only around 1% of the influent nitrogen was emitted as N_2O from the whole pilot plant.

By taking into account the biological parameters of the pilot plant, the nitrate concentration within the anoxic reactor turned out to be a key factor in the influence of the N_2O production. The experiments demonstrate that further studies are required in order to better understand the influence of the operational parameters, as well as of the biomass feature, in the N_2O production. Indeed, in the reported experiments a net contribution of the attached biomass or of the suspended biomass was not noticeable.

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