Greenhouse gases from membrane bioreactor treating hydrocarbon and saline wastewater

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Abstract

The effect of wastewater salinity and presence of petroleum hydrocarbon on N_2O emission was investigated in a membrane bioreactor, in which the anoxic and aerobic zones were put in series according to a pre-denitrification scheme. The pilot plant, was continuously fed by a mixture of real and synthetic wastewater. It was operated with a first phase of acclimation of the biomass to a given salinity by gradually increasing the salt concentration from 10 gNaCl/L to 20 gNaCl/L, and to a second phase of petroleum hydrocarbon dosing at 2 g/L (as gasoline). The first phase revealed a clear relationship between nitrous oxide emissions and salinity due to the increased NO_2-N production caused by the stress induced both on autotrophic and heterotrophic biomass by the increased salinity. However, after 45 days of operation, the growth rate of the autotrophic species started to recover, indicating acclimatization of the nitrifiers. In the second phase, the hydrocarbon shock induced a temporary complete inhibition of the biological activities, as well as the temporary suppression of the N_2O emissions. The observations in this study revealed that the oxic tank is the major source in terms of nitrous oxide emission flux. Indeed the aerobic tank emitted 1 or 2 order of magnitude more than the anoxic one. The reason of this is likely due to the stripping of the gas by aeration.

Introduction

Over the last decade, the interest in greenhouse gas (GHG) emissions from wastewater treatment plants (WWTPs) has significantly increased (GWRC, 2011; Law et al., 2012a). WWTPs can be considered as source of GHG emissions. Indeed, during wastewater treatment GHGs such as carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) can be directly emitted to the atmosphere contributing to the global warming (IPCC, 1996). More specifically, three main sources of GHG can be originated from a WWTP: direct, indirect internal and indirect external (GRP, 2008). Direct emissions of WWTPs are mainly related to biological processes (emissions of CO_2 from biomass respiration and/or N_2O from denitrification). Indirect internal emissions are associated with the consumption of purchased or acquired electricity, steam, heating or cooling. Finally, indirect external emissions are related to the overall sources not directly controlled inside the WWTP (e.g., sludge deposal or production of chemicals that are used in the plant). It is worth noting that, among the GHG produced, N_2O plays a major role in terms of climate change. In fact, it has a global warming potential about 300 times higher than that of CO_2, over a 100-year cycle. Therefore, even at small concentration it could have a strong impact on carbon footprint.

Law et al. (2012a) and de Haas and Hartley (2004) highlighted that a N_2O emission factor of 1.0% provides a carbon footprint comparable to that of the indirect carbon dioxide (CO_2) emission due to energy consumption in a conventional biological nutrient removal WWTP. Therefore, this means an increase of carbon footprint of a WWTP of about 30% (de Haas and Hartley, 2004).Thus, the understanding of the biological mechanisms involved in N_2O production during wastewater treatment is crucial in order to minimize the nitrous oxide emissions from WWTPs.

In the last years, there have been several attempts to understand the key factors affecting the GHG production processes in WWTPs (Daelman et al., 2012). Nevertheless, since the mechanisms that lead to N_2O production are process-specific and related to operating and environmental conditions, the data that have been published show a wide variation range of N_2O emission. Moreover, there is a lack of a standardized protocol for N_2O sampling and measurement from WWTPs.. A higher N_2O production has been observed, for instance, when performing biological treatment using synthetic rather than real wastewater; the reason of this being hypothesized being the lower biomass diversity when operating with synthetic wastewater (Yang et al., 2009).

However, although GHG emissions from WWTPs are nowadays of concern, several issues are still relatively unknown (Law et al., 2012a): GHG source and magnitude, referring in particular to N_2O; GHG magnitude from WWTPs treating industrial wastewater (e.g. wastewaters generated by washing oil tanks – slops). In this context, it is worth noting that N_2O emissions are mainly related to the processes associated with the biological nitrogen removal (Kampschreur et al., 2009). N_2O can be produced both during nitrification and denitrification processes (Kampschreur et al., 2008; Law et al., 2012b).

During nitrification, even if N_2O is not an intermediate in the main catabolic pathway, Ammonia Oxidizing Bacteria (AOB) are known to produce N_2O by two major mechanisms. The main contributor is the nitrifier denitrification through which nitrite is used as alternative electron acceptor to produce N_2O instead of being oxidized to NO_3 (Wrage et al., 2001, Law et al., 2012b). The other pathway is represented by the incomplete oxidation of hydroxylamine (NH_2OH) to NO_2 (Kampschreur et al., 2009, Chandran et al., 2011, Law et al, 2012a). Nitrifier denitrification by AOB is the predominant pathway in N_2O production especially under O_2 stress condition, which has been identified as the major factor leading to nitrous oxide emission (Kampschreur et al., 2009, Adouani et al., 2015). Specifically, when operating with low dissolved oxygen (DO) concentrations, Tallec et al. (2006) found that the 83% of the total nitrous oxide production could be ascribed to this pathway.

Additionally, increased N_2O production during AOB denitrification has been observed to have a strong correlation with NO_2 accumulation under either anoxic or aerobic conditions (Kampschreur et al., 2009, Yang et al., 2009, Yu et al., 2010).

During heterotrophic denitrification, N_2O is known to be an intermediate by-product of the process. The presence of a relatively high DO concentration in the anoxic reactor may inhibit the denitrification enzymatic activity; indeed, N_2O reductase is more sensitive to oxygen than other enzymes, leading as a consequence to N_2O

emission during denitrification (Kampschreur et al., 2009). Moreover, many other factors could influence the denitrification process and thus N_2O emission: among others, COD to N ratio, nitrite accumulation, typology of substrate and biomass, pH levels, temperature (Kampschreur et al., 2009, Law et al., 2011, Peng et al., 2014). Although nitrous oxide is an obligate intermediate product in the heterotrophic denitrification process, the primary emission source in a WWTP is represented by the aerobic zones. Indeed, the intensive aeration leads to N_2O stripping, promoting its emission in the environment.

Several attempts have been recently performed in order to increase the knowledge level and to identify the key elements affecting the nitrification process when treating industrial or saline wastewater, that can promote N_2O production and emission (Dvorak et al., 2013; Cortés-Lorenzo et al., 2015). Dvorak et al. (2013) investigated the nitrification process in a membrane bioreactor (MBR) system treating different percentage of industrial wastewater). They found no nitrification activity when the percentage of industrial wastewater fed to the MBR exceeded 50%. Cortés-Lorenzo et al. (2015) investigated the effect of salinity (expressed to as NaCl) at different concentrations on biological nitrogen removal and community structure of AOB species in a submerged fixed bed bioreactor. They found that ammonia oxidation activity significantly decreased and nitrite was consequently accumulated when the salt concentration was higher than 24.1 gNaCl L^{-1}. The nitrification inhibition could influence the N_2O production (Kampschreur et al., 2009). However, to authors' knowledge no studies have been yet performed with the aim to investigate the N_2O production when the nitrification process is hindered by high salt concentration in the wastewater.

To address the above mentioned knowledge gap, the N_2O formation mechanism in a MBR pilot plant has been investigated. The MBR pilot plant was designed for organic carbon and nitrogen removal from shipboard slop wastewater. The core features of this kind of wastewater are the high salinity level and high contents of hydrocarbons, deriving from tank washing. The MBR pilot plant was fed with a mixture of domestic and synthetic wastewater aimed at reproducing the features of real shipboard slops. The main aim of the study was to gain insight about the effect of hydrocarbons on N2O emissions both from oxic and anoxic tanks under high salinity

Materials and methods

The pilot plant (Figure 1) was built at the Laboratory of Environmental and Sanitary Engineering of Palermo University. It consisted of a feeding tank (volume 320 L) where real domestic wastewater was collected, two reactors in series, one anoxic (volume 45 L) and one aerobic (volume 224 L) according to the pre-denitrification scheme. Salt and gasoline were directly added into the anoxic tank. The solid-liquid separation was done by an ultrafiltration (UF) hollow fiber membrane module (Zenon Zeeweed, ZW 10, with specific area equal to 0.98 m^2 and nominal porosity of 0.04 μm). An oxygen depletion reactor (ODR) was installed in order to ensure the anoxic conditions inside the anoxic reactor despite the intensive aeration in the aerobic tank (Figure 1). The permeate extraction (Q_{OUT}) was imposed at 20 L h^{-1}. The aerobic, anoxic and MBR reactors were equipped with covering systems that enabled the gas accumulation into the head space, necessary for the consequent gas sampling. The pilot plant was started with activated sludge with a Mixed Liquor Suspended Solids (MLSS) concentration of 4,000 mg L^{-1} acclimated at 10 g NaCl/L. The experimental campaign had a duration of 88 days and was divided into two main phases: i. salinity acclimation at a given salinity; ii. hydrocarbon (gasoline) dosing. More specifically, during the first phase the biomass was acclimated to salinity by gradually increasing the salt concentration in the influent from10 gNaCl/L to 20 gNaCl/L. During the second phase, hydrocarbons at 20 mg TPH/L (TPH: Total Petroleum Carbon - as gasoline) concentration were added under the constant salinity of 20 gNaCl/l. The hydrocarbons concentration was chosen in order to simulate a shipboard slop already subjected to a physical-chemical pre-treatment.

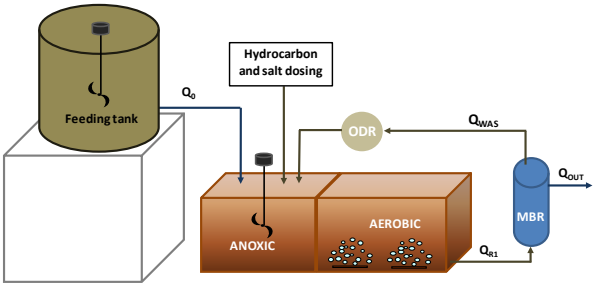

Figure 1. Layout of the pilot plant. Q_0 = influent wastewater; ODR = Oxygen Depletion Reactor; MBR = membrane Bioreactor; Q_{WAS} = recycled sludge from MBR to ODR; Q_{R1} = sludge feeding from aerobic tank to MBR.

In Table 1 the main wastewater characteristics as well as operational conditions are reported.

Parameter	Units	Value
COD	[mg L^{-1}]	350
TPH	[ppm]	20*
NH_4-N	[mg L^{-1}]	50
NaCl	[mg L^{-1}]	10-20
Permeate Flux	[L m^{-2} h^{-1}]	21
Flow rate	[L h^{-1}]	20
HRT	[h]	6

Table 1. Main characteristics of the feeding wastewater (on average) and operational conditions; *related only to the hydrocarbon dosing period

During plant operation, the influent wastewater, the mixed liquor inside the anoxic and aerobic tank and the effluent permeate was sampled and analyzed for total and volatile suspended solids (TSS and VSS), total chemical oxygen demand (COD_{TOT}), supernatant COD (COD_{SUP}) ammonium nitrogen (NH_4-N), nitrite nitrogen (NO_2-N),

nitrate nitrogen (NO_3-N), total nitrogen (TN), phosphate (PO_4-P), total carbon (TC) and inert carbon (IC). All analyses have been carried out according to the Standard Methods (APHA, 2005).

The pilot plant performance has been evaluated in terms of COD removal, nitrification/denitrification efficiency, nitrogen total removal and TPH removal. In order to discriminate the biological and physical contribution (due to the membrane), the COD removal efficiency has been distinguished between the COD removal inside the biological reactors and the overall COD removal (after membrane filtration). Specifically, the biological contribution has been evaluated as the difference between the influent COD_{TOT} value and the COD_{TOT} measured in the supernatant of mixed liquor samples withdrawn from the MBR tank. Conversely, the overall COD removal was assessed as the difference between the influent CODTOT and the permeate COD_{TOT} one. Respirometric batch experiments were periodically carried out during experiments using a "flowing gas/ static-liquid" type as batch respirometer (Spanjers et al., 1996). For the details on the adopted procedure, the reader is referred to literature (Di Trapani et al., 2014).

N_2O sampling and measurement

The monitoring of N_2O production was carried out in both anoxic and aerobic zones by withdrawing grab samples for both gas emitted and gas dissolved in the liquid phases.

The discrete samples of the gas emitted were collected by means of a syringe and a rubber septum inserted into the top of the tanks. Specifically in the anoxic reactor, the gas was withdrawn after headspace mixing by means of air injection in the headspace behind the tank covering. The gas was then injected into 7-mL sealed vials in order to guarantee several days of storage before laboratory measurements.

In order to study the possible temporal pattern of N_2O concentration, sampling operations have been performed every 10 minutes in a 2 hours sampling period. Three independent replicates were obtained for each grab sample.

The gas dissolved in the wastewater was determined by using the headspace gas method adapted from Kimochi et al. (1998). The adopted procedure consisted in centrifugation, at 8000 rpm for 5 min, of liquid samples, then a volume of 70 mL of supernatant (3 replicates were performed) was sealed into 125 mL of glass bottles together with 1 mL of 2N H_2SO_4 in order to prevent any biological reaction. A gentle stirring followed by 1 h without moving, allowed to reach the gas-liquid equilibrium. Thereafter, the gas accumulated in the bottle headspace, was collected and analized as a gas sample. The N_2O dissolved concentration was calculated using the Henry's Law. In this case, because of the higher complexity of the adopted method, a lower sampling frequency was used (1 sample per hour).

Moreover, the measurement of the gas advective flow was performed. First, by means of a hot-wire anemometer the gas velocity and the gas flow rate through the outlet section of the cover could be determined. Consequently, the N2O flux F (g m^{-2} h-1) from each surface was calculated according Equation 1.

$$F = \rho \cdot C \cdot Q/A \quad [1]$$

Where ρ (mol/m^3) is the density of the N_2O at the sampling temperature, C (mg/L) is the sample gas concentration, Q (m^3/h) is the total flow rate and A (m^2) is the total emissive surface area.

In order to promote the mixing in the headspace of the anoxic tank (Chandran, 2011), the flow rate measurement was obtained by injecting a sweep air flow rate (Q_{Sweep}) inside the reactor. Thus, the gas flow rate emitted from the anoxic tank was evaluated according to Equation [2].

$$Q_{gas} = v_{gas} \cdot A - Q_{Sweep} \quad [2]$$

The analysis was performed by using a Gas Chromatograph (Agilent Co. Ltd., USA) equipped with an Electron Capture Detector (ECD) and a Flame Ionization Detector (FID).

The comparison between the nitrous oxide production and nitrogen concentration in the several section of the MBR pilot plant was possible by calculating N_2O-N gas and dissolved concentration.

Results and discussion

Pilot plant performances

The main results in terms of average performances during the different stages of the experimental period are shown in Table 2.

The pilot plant showed a decrease of the biological COD removal (from 87% to the 64 %) with the increase of the salinity (Table 2). A rapid decrease of the biological COD removal occurred at 20 gNaCl L^{-1} (from 79 % to 63%) (Table 2). This result, as confirmed by the respirometric batch tests, is mainly related to the inhibition effect of the high salinity strength on the heterotrophic bacteria at 20 gNaCl L^{-1}.

In terms of overall COD removal efficiency the pilot plant showed very high performances throughout experimentals, with an average value close to 90%. In particular, during the period at 14 and 17 gNaCl L^{-1} the COD removal efficiency was higher than 96% (as average), confirming the effectiveness of the membrane process despite the high salinity (Di Bella et al., 2013; Jang et al., 2013). However, with the increase of the salinity, the reduction of the biological contribution entailed the total COD decrease (till to 75%) (Table 2). Nevertheless, in the phase with salinity at 20 gNaCl/L and hydrocarbons addition, the system showed a total COD removal equal to 91% (as average), thus confirming the key role exerted by membrane that compensated the poor biological efficiency (average value 64%) deriving from an inhibitory effect exerted by salinity and hydrocarbons.

The ammonia nitrification process was strongly influenced by the salinity increase. The average ammonia nitrification efficiency fluctuated in the range of 33-83% throughout the experiments (Table 2). The lowest ammonia nitrification efficiency (33%) was obtained at the highest salinity level (20 gNaCl L^{-1}) indicating the adverse effect of salt on the nitrification process. This result is in agreement with the literature experiences which suggest that nitrifiers are very sensitive to salinity load (Yogalakshmi and Joseph, 2010; Cortés-Lorenzo et al., 2015).

		Wastewater features				
		12 gNaCL L^{-1}	14 gNaCL L^{-1}	17 gNaCL L^{-1}	20 gNaCL L^{-1}	20 gNaCL L^{-1} + 20 mg TPH L^{-1}
Biological COD removal	[%]	87	81	79	63	64
Total COD removal	[%]	96	97	95	75	91
Nitrification	[%]	83	74	63	33	39
Denitrification	[%]	27	54	47	26	20
N removal	[%]	52	73	73	42	53
TPH removal	[%]	-	-	-	-	88

Table 2. Biological performance on average in terms of COD, N and TPH removal, nitrification and denitrification for each feeding wastewater feature

As reported in Figure 2, from 12 to 17 g NaCl L^{-1} the second nitrification step (from NO_2-N to NO_3-N) was partially inhibited and NO_2-N accumulation occurred inside the aerobic tank (ranging from 0.5 mg/L to 8 mg/L). As the salinity of the inlet wastewater was increased up to 20 g NaCl L^{-1} both the first and second nitrification steps were inhibited. Indeed, the concentration of NO_3-N and NO_2-N inside the aerobic reactor was equal to zero at the end of the period with 20 g NaCl L^{-1} (Figure 2). The hydrocarbon addition in the inlet influent leads to a further inhibition effect on nitrifier activity, also confirmed by the result of the respirometric batch tests. Indeed, with the hydrocarbon addition both NO_3-N and NO_2-N concentration inside the aerobic tank persisted to be zero for almost 30 days (Figure 2). After that a recovery of the nitrification process occurred as confirmed by the increase of NO_3-N concentration inside the aerobic tank (Figure2).

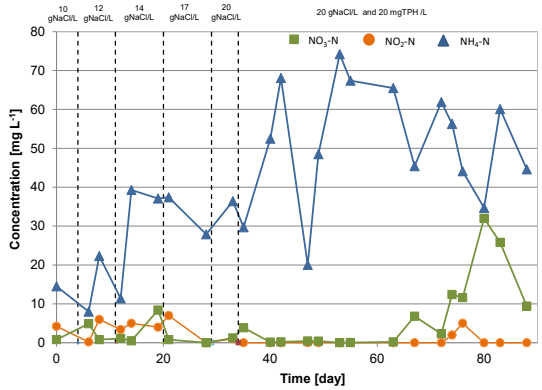

Figure 2 Influent NH_4-N concentration, NO_2-N and NO_3-N concentration in the aerobic tank

Concerning the denitrification process very low efficiency were found over the experimental period (ranging between 27 and 54%). This result was debit two twofold factors: i. inability to maintain constantly null the dissolved oxygen concentration inside the anoxic tank; ii. inhibition effect of the salt on heterotrophic bacteria.

Finally, in terms of TPH a quite high (88%) removal efficiency was obtained during the experimental period. This is indicating that MBR may be a promising technology for treating oily wastewater.

Biomass biokinetic behaviour

Referring to heterotrophic activity, Figure 3 shows the trend of specific OUR (SOUR) rates during experiments. It is worth noting that it was observed a significant decrease of biomass respiration rates likely due to a stress effect due to the presence of hydrocarbons in the inlet wastewater. Moreover, the heterotrophic biomass showed a "storage" phenomenon, typical of systems subjected to dynamic conditions. This situation likely enhanced the growth of bacterial groups able to rapidly convert the organic substrate into storage products.

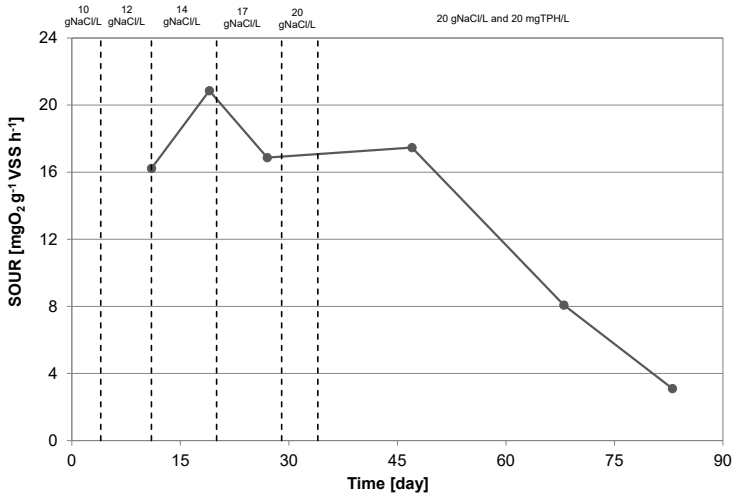

Figure 3. Specific respiration rates of heterotrophic species

Referring to nitrification, in the first portion of experiments, a significant inhibition of autotrophic species was observed, mostly due to the effect of salinity (Figure 4). Indeed, autotrophic species have been recognized to be very sensitive to salt variations (Di Trapani et al., 2014). However, after experimental day 45, a considerable increase of autotrophic growth rates was noticed, highlighting a recovering of nitrification. This result is likely related to the acclimation of autotrophic species, suggesting that in the long term it is possible to restore a good nitrification ability of the system, even in presence of moderate to high saline concentration and presence of petroleum hydrocarbon content as in slop wastewater.

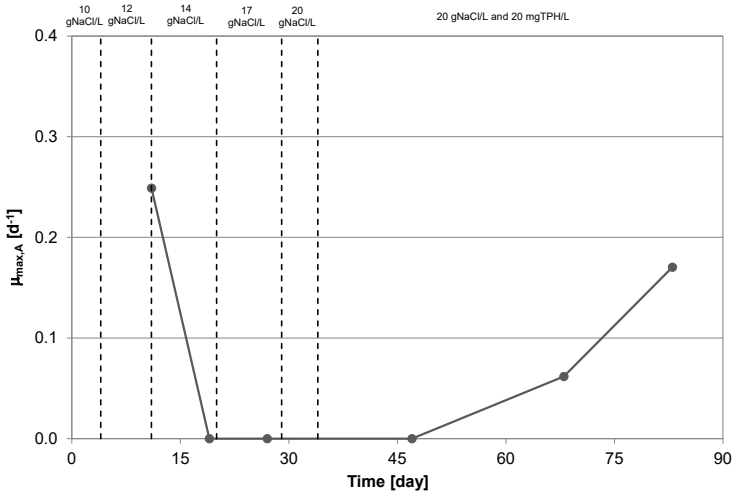

Figure 4. Maximum growth rates for autotrophic species

N_2O emission trend

Figure 5 reports the N_2O-N concentration (Figure 5a) as well as the N_2O-N flux (Figure 5b) emitted from aerobic and anoxic tank throughout experiments.

In terms of concentration (Figure 5a), an increase of N_2O-N production with the increase of salinity was observed. At 20 gNaCl/l the NO_2-N concentration in both aerobic and anoxic tank was around 40% higher than at the 17 gNaCl/l condition (Figure 5a). This result is in agreement with previous experiences, when a significant correlation between salinity and N_2O production/emission was established (Tsuneda et al., 2005; Mannina et al., 2015).

Moreover, a moderate predominance of N_2O-N concentration in the anoxic tank was observed. It is likely due to higher inhibition effect of salinity on autotrophic bacteria than heterotrophic one and to the dilution effect (close to 30%) exerted by the aeration in the aerobic tank.

The strong nitrification inhibition when the salt concentration gradually increased from 12 to 20 gNaCl/L led to a greater NO_2-N production in the anoxic tank than the aerobic.

From the 40th experimental day until the day 76, it was observed a significant decrease of N_2O-N production (Figure 5a). This result could likely be related to the addition of hydrocarbons in the influent wastewater; indeed when hydrocarbons were fed into the MBR pilot plant, it was observed a drastic inhibition of both nitrification processes. This result was also confirmed by the respirometric batch tests that highlighted a negligible autotrophic activity and an inhibition of heterotrophic bacteria in the same period. This confirm that when the nitrification and denitrification ability are almost supressed also the N_2O-N emissions fall significantly down; this results

is in agreement with Tsuneda et al. (2005). Finally, at the end of the experimental period, the nitrification activity was recovered and with it the nitrous oxide production; in detail, referring to day 80 of the experimental campaign, the N_2O-N concentration in the gas phase was respectively 30 and 76 times higher than that measured in the previous sampling day (Figure 5a). Indeed, the shock hydrocarbon addition together with high salinity induced a temporary inhibition of the biomass, which was overcome once being acclimated.

Referring to the N_2O-N flux (Figure 5b), higher values were emitted from the aerobic tank, since the intensive aeration might lead to N_2O-N stripping as suggested by Law et al. (2012b). The N_2O-N flux trend has the same pattern of the N_2O-N concentration one from day 35 to day 75. It is worth noting that the flux emitted from the aerated tanks is 1 or 2 order of magnitude higher than that emitted from the anoxic one (Yang et al., 2009; Law et al., 2012b; Daelman et al., 2013).

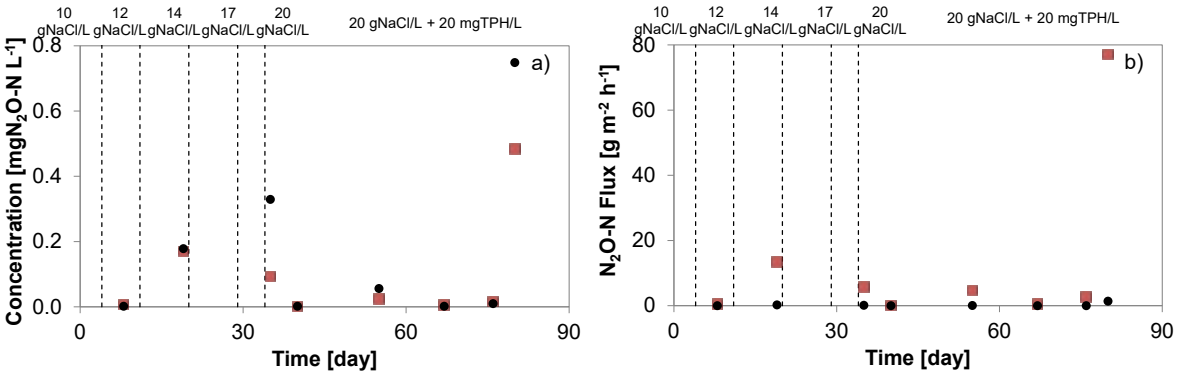

Figure 5 N_2O concentration (a) and N_2O flux (b) in aerobic (■) and anoxic tank (●)

As discussed above, one of the main effect of salt and hydrocarbon addition was the partial nitrification and denitrification. This result is in line with previous studies (Kampschereur et al., 2009) and led to different nitrogen pathways, thus producing N_2O. Figure 6 shows the nitrous oxide concentration vs the denitrification efficiencies. From Figure 6 it is possible to observe that, as soon as the denitrification efficiency is low, the N_2O-N concentration is high. As matter of the fact, there is a good correlation between N_2O_N and denitrification efficiency, with a correlation coefficient R^2 equal to 0.98.

Inside the anoxic tank, where N_2O-N is an intermediate of the sequential reduction of NO_3-N to N_2 gas, has been observed a remarkable correlation with the denitrification efficiency, as shown in Figure 6. The outliers are the sampling related to the experimental day during which the biomass was subjected to the hydrocarbon and high salinity shock (phase at 20 g NaCl/l and 20 mg TPH/L).

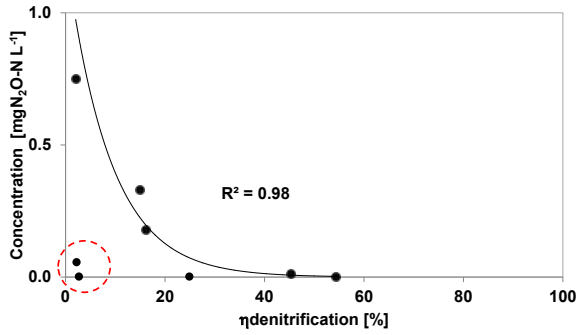

Figure 6 Nitrous oxide and biological efficiency in the anoxic tank; the red circles highlight the point excluded by the correlation

For sake of completeness in Figure 7 a typical pattern of N_2O-N concentration during a sampling day, both in the aerobic and anoxic tank, is reported. In detail, the figure is referred to the 35th experimental day, when salinity and hydrocarbon concentration were 20 gNaCl L^{-1} and 20 mgTPH L^{-1} respectively. By analysing Figure 7 it is possible to observe a slight predominance of N2O production in the anoxic phase compared to the aerobic one, moreover the nitrous oxide produced was almost constant during the whole sampling day.

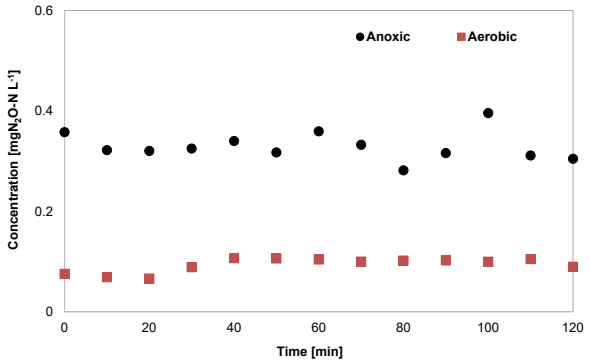

Figure 7 N_2O-N concentration during the 35th experimental day in aerobic and anoxic tank

Regarding the NO_2-N concentration in the liquid phase, the average value measured during the sampling period is 0.18 mg N_2O-N L^{-1} in the aerobic tank and 0.22 mg N_2O-N L^{-1} in the anoxic one.

Influence of nitrite accumulation on N_2O emission

Figure 8 reports the relationship between nitrite and N_2O concentration in the aerobic (Figure 8a) and anoxic (Figure 8b) tank. By analysing Figure 8, one can observe a good correlation between nitrite accumulation in the liquid phase and N_2O-N emission. Important to precise is that data related to the days of the complete inhibition of autotrophic bacteria (first 35 days at 20 gNaCl/L and 20 mgTPH/L) have been excluded from the correlation reported in Figure 8 (highlighted by the red circle).

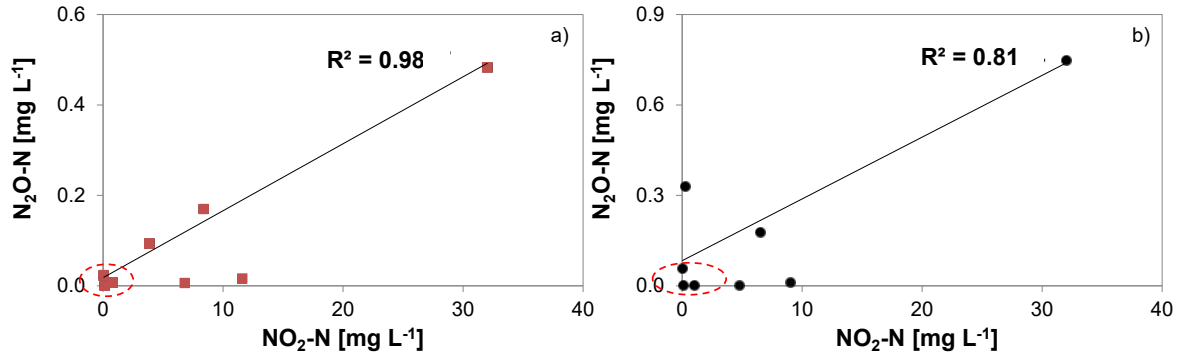

Figure 8 N_2O-N concentration and nitrite in aerobic (a) and anoxic (b) tanks, the red circles highlight the point excluded by the correlation

Results reported in Figure 8 show a linear dependency between the NO_2-N accumulation and the N_2O-N production both in the aerobic and anoxic tank. Thus, corroborating the results found in literature that identify the NO_2-N concentration as a key factor able to suggest the potential N_2O-N production (inter alia, Tsuneda et al., 2005; Yang et al., 2009; Kampschereur et al., 2009).

Conclusions

In this study, the effect of salinity and hydrocarbon dosage on N_2O-N emission in and MBR pilot plant treating saline wastewater was investigated. The following conclusions were drawn:

- The total COD removal efficiency was not affected by the salinity (up to 17 g NaCl/l) and petroleum hydrocarbon dosage (20 g gasoline/l) inside the system, confirming the effectiveness of the MBR process to treat high strength salinity wastewater.
- A significant decrease of heterotrophic respiration rates was observed due to a stress effect of petroleum hydrocarbons (gasoline) in the inlet wastewater. Furthermore, a significant inhibition of autotrophic species, mostly due to the effect of salinity was found at 20 gNaCl/L and 20 mgTPH/L of the inlet wastewater. Finally, the acclimation of autotrophic species occurred and the nitrification process after 45 days.
- The biological stress induced by the salinity level promote the increase of the N_2O emission from the aerobic and anoxic tanks in a pre-denitrification system.
- The hydrocarbon shock together with the high salt concentration led to a temporary complete inhibition of the biological activity, at the same time the nitrous production fall down. After that, nitrification and denitrification ability were recovered as the N_2O emission.
- A significant relationship between N_2O emission and nitrite dissolved in the liquid phase was found, confirming that the accumulation of NO_2 is a key factor that promote the nitrous oxide production.

- The autotrophic denitrification by AOB in the oxic tank is revealed to be the major source in terms of nitrous oxide emission flux. The reason of this is likely due to the stripping of the gas by aeration.

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