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Nitrous oxide emissions in a membrane bioreactor treating saline wastewater contaminated by hydrocarbons

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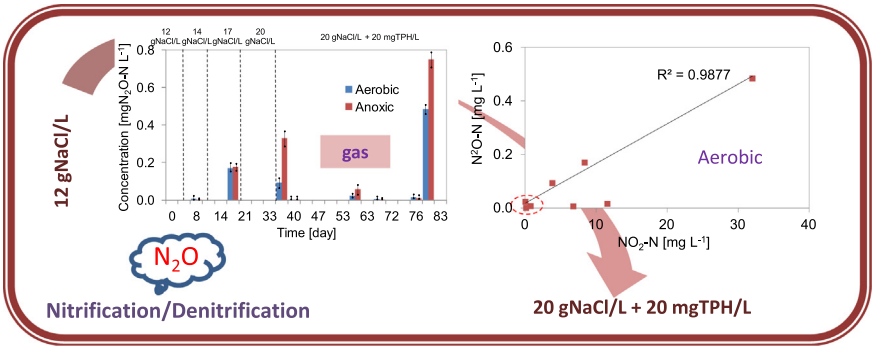
HIGHLIGHTS

- N_2O production in a MBR with salinity variation and hydrocarbon dosage was studied.
- The biological stress induced by the salinity promotes the N_2O emissions.
- The hydrocarbon shock led to a temporary fall down of N_2O production.
- Significant relationship between N_2O emission and nitrite in the liquid phase.
- Autotrophic denitrification by AOB was the major source of N_2O emission.

GRAPHICAL ABSTRACT



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ABSTRACT

The joint effect of wastewater salinity and hydrocarbons on nitrous oxide emission was investigated. The membrane bioreactor pilot plant was operated with two phases: i. biomass acclimation by increasing salinity from 10 gNaCl L^{-1} to 20 gNaCl L^{-1} (Phase I); ii. hydrocarbons dosing at 20 mg L^{-1} with a constant salt concentration of 20 gNaCl L^{-1} (Phase II). The Phase I revealed a relationship between nitrous oxide emissions and salinity. During the end of the Phase I, the activity of nitrifiers started to recover, indicating a partial acclimatization. During the Phase II, the hydrocarbon shock induced a temporary inhibition of the biomass with the suppression of nitrous oxide emissions. The results revealed that the oxic tank was the major source of nitrous oxide emission, likely due to the gas stripping by aeration. The joint effect of salinity and hydrocarbons was found to be crucial for the production of nitrous oxide.

1. 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). 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 global warming (IPCC, 1996). Among the GHGs produced, N_2O plays a major role in terms of climate change. In fact, it is characterized by a global warming potential about 300 times higher than that of CO_2, over a 100-year cycle. Therefore, even for small production amounts it might have a strong impact on carbon footprint. As a result, N_2O emissions from wastewater treatment has received increasing attention in recent years (Ni and Yuan, 2015; Mannina et al., 2016a).

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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 that the WWTP carbon footprint will increase 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, several attempts have been devoted to understand the key factors affecting N_2O production pathways 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 literature data 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. Indeed, it was hypothesized that this result was related to 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/or 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 AOB denitrification through which nitrite is used as alternative electron acceptor to produce N_2O instead of being further 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). AOB denitrification 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, the 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, 2015). 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 into 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; Liu 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. Cortés-Lorenzo and co-authors found a remarkable decrease of the ammonia oxidation activity as well as a significant inhibition of the system nitrification ability, leading to nitrite accumulation when the salt concentration was higher than 24.1 gNaCl L^{-1}, due to a severe inhibition of *Nitrite Oxidizing Bacteria* (NOB). The nitrification inhibition could influence the N_2O production (Kampschreur et al., 2009). Liu et al. (2015) investigated the effect of salinity on N_2O emission during leachate landfill treatment. Liu and co-workers found that when salinity was increased from 10 to 35 g L^{-1} the removal efficiency of NH_4-N removal dropped from 99.3 to 83.9% promoting at the same time NO_2-N accumulation and the N_2O emissions.

However, most of the literature findings are often based on conventional activated sludge (CAS) processes and to authors' knowledge no studies have been yet performed with the aim to investigate the short term N_2O production in membrane bioreactors (MBRs) under the joint effect of salinity and hydrocarbons. Indeed, the biological consortium of MBRs might be characterized by specific peculiarities that can promote the N_2O production/emission. Moreover, the knowledge acquired in the technical literature is often based on batch experiments with only few analyses of continuous-fed dynamic plants. This can be of huge importance and requires further exploration. Indeed, there is an imperative need of long terms analysis in order to take into account the evolution of the biomass properties over the time and their influence in N_2O formation (Daelman et al., 2015; Sperandio et al., 2016).

Bearing in mind these considerations, the aim of the study was to investigate the joint effect of salt and hydrocarbons (as diesel fuel) in the short term on N_2O production from a MBR pilot plant conceived for organic carbon and nitrogen removal from shipboard slop wastewater. The core features of this 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 already subjected to a physical-chemical pre-treatment. The N_2O emissions from both aerobic and anoxic compartments were assessed, in order to elucidate the main mechanisms of N_2O formation when treating this kind of wastewater.

The newness appealing of the proposed study relies on the following considerations: complexity of the investigated system (biological process for carbon and nitrogen removal coupled to membrane filtration unit), long-term operations and real wastewater fed to the MBR pilot plant.

2. Materials and methods

2.1. The MBR pilot plant

The MBR pilot plant (Fig. 1) was built at the Laboratory of Environmental and Sanitary Engineering of Palermo University and was designed according to the modified Ludzack-Ettinger (MLE) scheme. It consisted of a feeding tank (volume 320 L) where

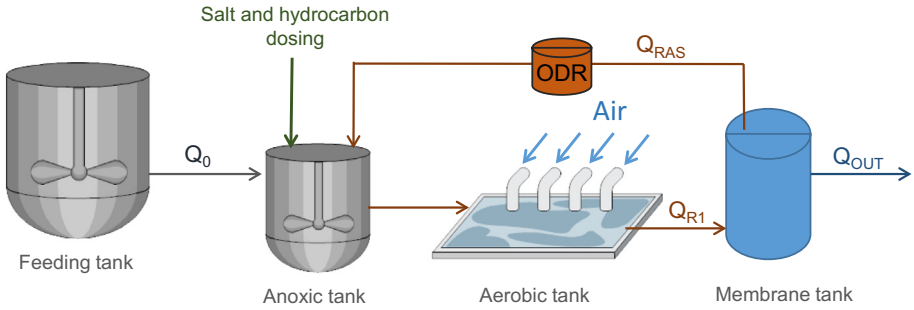

Fig. 1. Layout of the pilot plant. Q_0 = influent wastewater; ODR = oxygen depletion reactor; Q_{RAS} = recycled sludge from MBR to ODR; Q_{R1} = sludge feeding from aerobic tank to MBR.

real domestic wastewater was collected, two reactors in series, one anoxic (volume 45 L) and one aerobic (volume 224 L) according to a pre-denitrification scheme. Salt and hydrocarbon were directly added into the anoxic tank through a dosing pump, in order to avoid mixing issues in the feeding tank. The solid-liquid separation was carried out 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 (Fig. 1). The permeate extraction (Q_{OUT}) was imposed at 20 L h^{-1} and the permeate flux was maintained equal to 21 L m^{-2} h^{-1}, with an influent flow rate (Q_0) of 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 up with activated sludge inoculum at a Mixed Liquor Suspended Solids (MLSS) concentration of 4 g L^{-1} acclimated at a feeding salt rate of 10 g NaCl L^{-1}. The acclimation period (from 0 to 10 gNaCl L^{-1}) had an overall duration of 85 days (Mannina et al., 2016d). The MBR pilot plant was operated with hydraulic retention time (HRT) of 16 h.

2.2. Experimental campaign and influent features

The experimental campaign had a duration of 90 days and was divided into two main phases. In the Phase I, the biomass was acclimated to a gradual increase of the feeding salt rate and lasted 45 days; in the Phase II, hydrocarbons were dosed as diesel fuel and lasted during days 45–90. More specifically, during the Phase I the biomass was acclimated to salinity by gradually increasing the salt concentration in the influent from 10 gNaCl L^{-1} to 20 gNaCl L^{-1}. During the Phase II, hydrocarbons at 20 mg TPH L^{-1} (TPH: Total Petroleum Hydrocarbon) concentration were added under the constant salinity of 20 gNaCl L^{-1}. The hydrocarbons concentration was chosen in order to simulate a shipboard slop already subjected to a physical-chemical pre-treatment. The average influent COD and NH_4-N were equal to 350 mg L^{-1} and 50 mg L^{-1}, respectively. Table 1 summarizes the average characteristics of the feeding wastewater as well as plant operational features in the Phase II.

2.3. Analytical methods

N_2O concentration was measured by using a Gas Chromatograph (Thermo Scientific™ TRACE GC) equipped with an Electron Capture Detector.

Influent wastewater, mixed liquor inside the biological tanks (anoxic and aerobic) and effluent permeate were monitored in terms of total chemical oxygen demand (COD_{TOT}), soluble COD (COD_{SOL}), 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), inert carbon (IC). The measurements have been carried out according to Standard Methods (APHA, 2005). It is worth noting that liquid and gaseous samples were collected simultaneously. The TPH content was measured on extracted and evaporated samples. An AGILENT 6890 GC-FID system with autosampler and a Parker gas generator 9090 for hydrogen supply was adopted.

Table 1
Average features of the feeding wastewater and main operational conditions during experiments.

Parameter	Units	Phase I	Phase II
COD	[mg L^{-1}]	340	610
TPH	[mg L^{-1}]	–	20
NH_4-N	[mg L^{-1}]	25	50
TN	[mg L^{-1}]	32	56
NaCl	[mg L^{-1}]	10–20	20
Permeate Flux	[L m^{-2} h^{-1}]	21	21
Flow rate	[L h^{-1}]	20	20
HRT	[h]	16	16
DO anoxic	[mg L^{-1}]	0.25	0.87
DO aerobic	[mg L^{-1}]	2.4	1.63

2.4. Pilot plant removal performances

The MBR pilot plant performance has been evaluated in terms of COD removal, nitrification/denitrification efficiency, nitrogen total removal and TPH removal. In order to discriminate between the removal effect of the biological processes and the filtration operated by the membrane, two different removal efficiencies have been calculated: the biological removal efficiency and the total removal efficiency. The former was calculated as the difference between the total COD (COD_{TOT}) value in the influent and the COD_{SOL} measured in the supernatant of mixed liquor samples (filtered at 0.45 μm) withdrawn from the MBR tank ($COD_{SOL,MBR}$). Conversely, the total COD removal efficiency (also including the effect of the removal effect of the membrane filtration) was assessed as the difference between the COD_{TOT} in the influent and in the permeate.

Nitrification (η_{nit}), denitrification (η_{denit}) and nitrogen (η_{total}) removal efficiencies were evaluated according to the following expressions (Mannina et al., 2016e; Wagner et al., 2015):

$$\eta_{nit}(\%) = \frac{(NH_4^+ - N_{in}) - (NH_4^+ - N_{out}) - N_{assimilation}}{(NH_4^+ - N_{in}) - N_{assimilation}} \quad (1)$$

$$\eta_{denit}(\%) = \frac{(NH_4^+ - N_{in}) + (NO_x - N_{in}) - (NH_4^+ - N_{out}) - N_{assimilation} - (NO_x - N_{out})}{(NH_4^+ - N_{in}) + (NO_x - N_{in}) - N_{assimilation}} \quad (2)$$

$$\eta_{total}(\%) = \frac{(NH_4^+ - N_{in}) + (NO_x - N_{in}) - (NH_4^+ - N_{out}) - (NO_x - N_{out})}{(NH_4^+ - N_{in}) + (NO_x - N_{in})} \quad (3)$$

where NH_4^+-N_{in} = influent nitrogen ammonia concentration; NH_4^+-N_{out} = permeate nitrogen ammonia concentration; $N_{assimilation}$ = assimilated nitrogen (as 5% of the total BOD removed) NO_x-N_{in} = influent nitrite and nitrate concentration; NO_x-N_{out} = permeate nitrite and nitrate concentration.

The TPH removal efficiency was assessed as the difference between the influent TPH and the TPH measured in the permeate samples.

2.5. Gas sampling

Liquid and gaseous samples were withdrawn from the aerobic and anoxic tanks and analysed for the evaluation of N_2O concentration. Furthermore, the N_2O fluxes (gN_2O-N m^{-2} h^{-1}) emitted from both the aerobic and anoxic compartments were quantified by measuring the gas flow rates Q_{gas} (L min^{-1}) from the aerobic and anoxic tanks, as better outlined in the following Sections 2.5.1 and 2.5.4.

2.5.1. Gas flow rate measurement

Q_{gas} was indirectly evaluated according to the following Eq. (4):

$$Q_{gas} = v_{gas} \cdot A \quad (4)$$

where A represents the outlet section (m^2) and v_{gas} (m s^{-1}) is the gas velocity, measured by using the TMA-21HW – Hot Wire anemometer. During the flow rate measurements in the anoxic compartment a sweep air flow rate (Q_{Sweep}) was supplied inside the reactor in order to promote the gas mixing and to facilitate the gas sampling at low gas flow rate condition (Chandran et al., 2011). Thus, the gas flow rate emitted from the anoxic tank was evaluated according to Eq. (5).

$$Q_{gas} = v_{gas} \cdot A - Q_{sweep} \quad (5)$$

2.5.2. Gas phase sampling

Gas samples were withdrawn by means of commercial syringes and transferred into glass vials (e.g., LABCO Exetainer, 738 model) where the vacuum was previously created.

In order to guarantee the atmospheric pressure inside the vials, the ratio between the volume of the gas sample (inserted inside the vial) and the volume of the vial has to be not less than 1.25 (e.g., 15 mL of sample in vial of 12 mL).

Grab samples were collected every 15 min in a 3 h sampling period (total duration of each cycle). Three replicates were carried out for each grab sample. The N_2O concentration was then calculated as the average value among the 3 replicates.

2.5.3. Dissolved gas sampling

Dissolved gas sampling was carried out on the basis of the head space gas method derived from Kimochi et al. (1998). Briefly, 70 mL of supernatant (after 5 min centrifugation at 8000 rpm) were sealed into 125 mL glass bottles. In order to prevent any biological reaction, 1 mL of 2 N 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 head space of the bottles was collected similarly to the gas sampling procedure.

Finally, by applying the Henry's Law, the dissolved gas concentration at the equilibrium with the headspace gas was calculated. In this case, a lower sampling frequency was used (1 sample per hour).

2.5.4. Gas flux quantification

The i-th gas flux (F_i) emitted from the j-th tank was quantified according to the Eq. (6) derived from Yan et al. (2014).

$$F_i = \rho_i \cdot C_i \cdot \frac{Q_{gas,j}}{A_j} \quad (6)$$

where, ρ_i (mol m^{-3}) is the density of the i-th gas at the record temperature, C_i (mg L^{-1}) is the i-th gas concentration during the sampling period; $Q_{gas,j}$ (L min^{-1}) is the gas flow rate emitted from the j-th tank; A_j (m^2) represents the emitted surface of the j-th tank.

2.6. N_2O emission factors

For both the aerobic and anoxic compartment, the evaluation of the N_2O emission factors, expressed as the percentage of N_2O emitted compared to the inlet nitrogen loading rates, was carried out by means of the following expression derived by Tsuneda et al. (2005):

$$EF_{N_2O} = \frac{N_2O_{Gas}/\Delta t + N_2O_{Dissolved}/HRT}{TN_{IN}/HRT} \quad (7)$$

where EF_{N_2O} is the emission factor, N_2O_{Gas} is the nitrous dioxide in the gaseous phase, $N_2O_{Dissolved}$ is the nitrous dioxide in the liquid phase, Δt is the time between nitrogen gas replacement and gas sampling TN_{IN} is the influent total nitrogen concentration while HRT is the hydraulic retention time of the MBR pilot plant.

2.7. Evaluation of biomass respiratory activity

Respirometric batch experiments were periodically carried out during experiments using a "flowing gas/static-liquid" type as batch respirometer. The biomass samples were transferred to the respirometer and optionally diluted with permeate, if necessary, in order to achieve a volatile suspended solid (VSS) concentration in the range of 2.0–3.0 g L^{-1}. Before running the respirometric test, each sample was aerated until endogenous conditions were reached, basing on the monitoring of the oxygen uptake rate (OUR) values. In the batch tests aimed at evaluating the heterotrophic biokinetic parameters, the nitrifying biomass was inhibited by adding 10 mg L^{-1} of Allylthiourea (ATU), whilst the exogenous OUR was enhanced by the addition of a readily biodegradable organic substrate (sodium acetate in the present study). The estimation of the kinetic parameters for the autotrophic species was carried out with the same procedure. However, no inhibiting substance like ATU was added and ammonium chloride (NH_4Cl) was spiked to enhance the exogenous OUR values and the calculation of the biokinetic parameters. For further details on the adopted protocol, the reader is referred to literature (Mannina et al., 2016b).

3. Results and discussion

3.1. Pilot plant performances

Fig. 2 shows the results of the MBR pilot plant performance in terms of COD (Fig. 2a) and nitrogen removal efficiencies (Fig. 2b). More specifically, Fig. 2a shows the trend of the total and biological COD removal efficiency, whereas Fig. 2b reports the pattern of η_{nit}, η_{denit} and ηN_{total}.

During the Phase I, the MBR pilot plant showed a decrease of the biological COD removal (from 87% to 63%) with the increase of salinity (Fig. 2a). Moreover, a significant and fast decrease of the biological COD removal occurred when the salt concentration

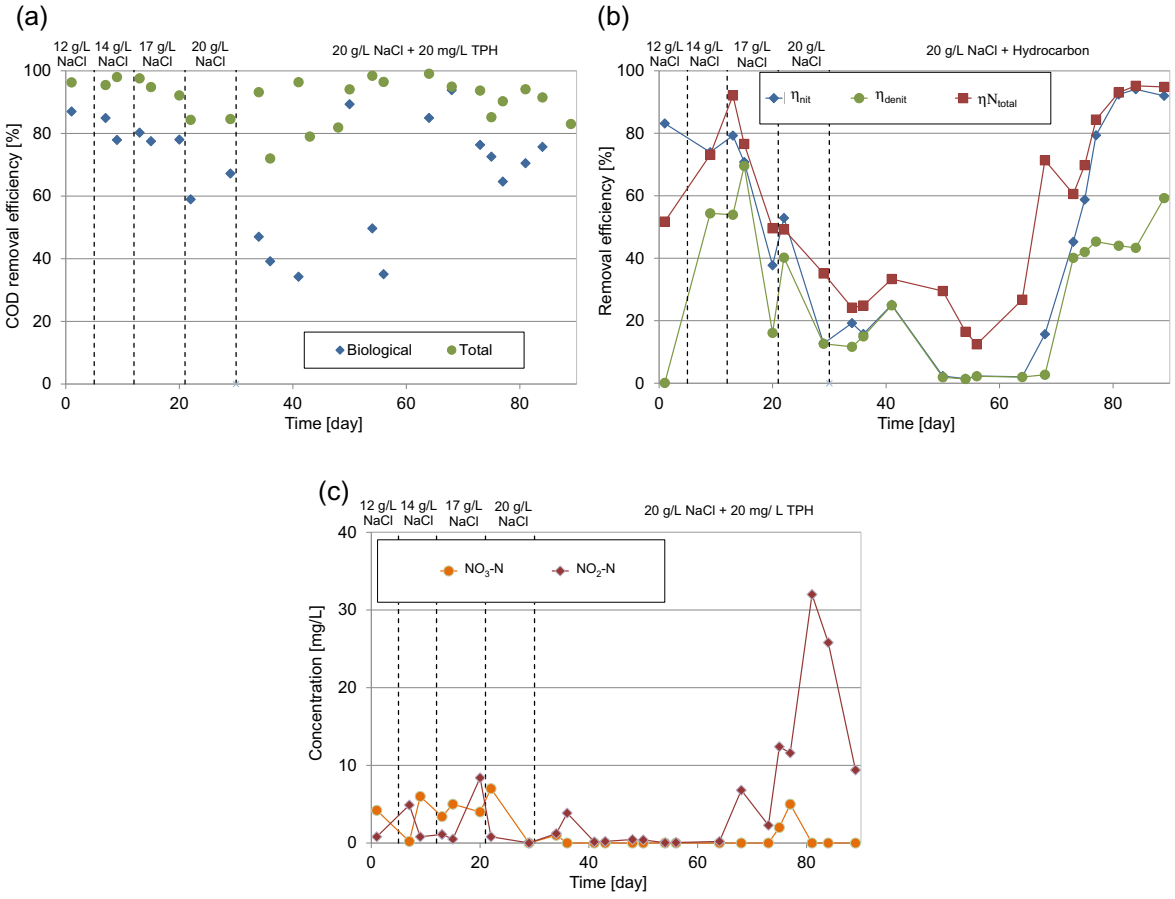

Fig. 2. Pattern of the COD removal efficiencies (a); pattern of the nitrification, denitrification and total nitrogen removal efficiencies (b); NO_2-N and NO_3-N concentration in the aerobic tank (c).

was increased up to 20 gNaCl L^{-1} (from 79% to 60%) (Fig. 2a). This result suggests that a feeding salt rate of 20 gNaCl L^{-1} may represent a sort of threshold value inducing a strong inhibition on the heterotrophic bacteria activity in the short term. Nevertheless, in the long term the system might recover from the inhibition.

In terms of total COD removal efficiency, the pilot plant showed very high performances throughout the two experimental phases, with an average value close to 90% (Fig. 2b). 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 (Jang et al., 2013). However, with the increase of the salinity, the reduction of the biological efficiency entailed the total COD decrease (till to 75%) (Fig. 2a). Nevertheless, during the Phase II (with salinity at 20 gNaCl L^{-1} 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 the inhibitory effect exerted by salinity and hydrocarbons. This result was confirmed by the respirometric batch tests as better outlined in the following Section 3.2. In terms of TPH, a removal efficiency of 88% was obtained during the experimental period. The nitrification process was strongly influenced by the salinity increase. The average nitrification efficiency fluctuated in the range of 33–83% throughout experiments (Fig. 2b). The lowest nitrification efficiency was obtained during the Phase II indicating the adverse effect of high feeding salt rate (20 g NaCl L^{-1}) on the activity of autotrophic species. This result is in agreement with the literature data, which suggest that nitrifiers are very sensitive to the variation of salinity loading rate (Yogalakshmi and Joseph, 2010; Cortés-Lorenzo et al., 2015). Concerning the denitrification process, the removal efficiencies achieved throughout experiments ranged between 2 and 72%. The lowest denitrification efficiency was obtained during the Phase II due to the strong inhibition of the biological processes promoted by salt and hydrocarbons concentration (Fig. 2c). Indeed, by analysing Fig. 2c one may observe that NO_2-N accumulation (from 0.5 mg L^{-1} to 8 mg L^{-1}) occurred inside the aerobic reactor from 12 to 17 g NaCl L^{-1}. This result highlighted that the NOB activity was severely affected by the salinity variation, confirming that NOB microorganisms are highly sensitive to the feeding salt rate. Conversely, both AOB and NOB species were severely inhibited at 20 g NaCl L^{-1} with NO_3-N and NO_2-N concentrations that decreased close to zero inside the aerobic reactor (Fig. 2c).

The hydrocarbon dosage contributed to a further inhibition of nitrifiers activity producing an almost complete suppression of the nitrification, also confirmed by the NO_3-N and NO_2-N concentrations (almost null) inside the aerobic tank (Fig. 2c). Nevertheless, in the last days of the Phase II (after experimental day 67), the NO_2-N started again to accumulate inside the aerobic tank while NO_3-N production remained quite negligible (Fig. 2c). This result suggests that a recovery of AOB activity occurred, whereas NOB species were still inhibited by high saline environment and that longer durations would be likely necessary to recover NOB activity (Fig. 2c).

For further details about the system performance as well as the nitrogen balance, the reader is referred to literature (Mannina et al., 2016b).

3.2. Biomass respiratory activity

During experiments, a significant decrease of biomass respiration rates was experienced after hydrocarbons addition to the inlet wastewater. Indeed, the specific oxygen uptake rate (SOUR) values (as average) decreased from 17, 99 to 9.55 mg O_2 g^{-1} TSS h^{-1} in the Phase I and II, respectively. Nevertheless, it is worth mentioning that the decrease of the OUR values was delayed compared to the starting day of hydrocarbons dosage, thus suggesting that the harmful effect on heterotrophic activity could be mainly due to the accumulation of hydrocarbons inside the system.

On the other hand, the autotrophic species highly suffered the highest salt concentrations of the Phase I (17 and 20 g NaCl L^{-1}) that caused a huge worsening of the nitrifying activity, with maximum growth rate ($\mu_{A,max}$) and nitrification rate that decreased close to zero. This result is in good agreement with previous studies that outlined the high sensitivity of autotrophic species to salt variations (Mannina et al., 2016d). For further details on the respirometric results as well as on biokinetic/stoichiometric parameters, the reader is addressed to literature (Mannina et al., 2016b).

3.3. Gaseous N_2O-N concentration and emission

Fig. 3 reports the average gaseous N_2O-N concentration (Fig. 3a) as well as the N_2O-N flux (Fig. 3b) emitted from the aerobic and anoxic tanks throughout experiments. For sake of completeness, Fig. 3c shows a typical pattern of N_2O-N concentrations during a sampling day, referring to both the aerobic and anoxic tank.

In terms of gaseous concentration (Fig. 3a), it was observed an increase of N_2O-N production with the increase of salinity. Indeed, at 20 gNaCl L^{-1} (Phase II) the N_2O-N emission in both aerobic and anoxic tank was around 40% higher than at 17 gNaCl L^{-1} (Fig. 3a). This result is in agreement with previous experiences where a significant correlation between salinity and N_2O production/emission was found (Tsuneda et al., 2005; Mannina et al., 2016c).

This result might be related to the inhibition of the nitrification process (referring in particular to NOB activity) when the salt concentration gradually increased from 12 to 20 gNaCl L^{-1} that could promote a higher N_2O-N production (Fig. 3a).

Moreover, a moderate predominance of N_2O-N concentration in the anoxic tank was observed (Fig. 3a). Such a result is consistent with previous findings that identified the anoxic compartment as a major source of N_2O-N emission (Kampschreur et al., 2009). Indeed, since N_2O represents an intermediate product of the heterotrophic denitrification process, the incomplete denitrification might promote N_2O accumulation and emission.

From the 40th experimental day until the day 76, it was observed a significant decrease of N_2O-N production (Fig. 3a). This result could likely be related to a huge inhibition of the biological performance of the system, also emphasized by the addition of hydrocarbons in the influent wastewater. Indeed, when hydrocarbons were fed to the MBR pilot plant, it was observed a drastic inhibition of both NOB and AOB species. This result was also confirmed by the respirometric batch tests that highlighted a negligible autotrophic activity and an inhibition of heterotrophic bacteria during the same period (Mannina et al., 2016b). This result confirmed that when the nitrification and denitrification ability are almost suppressed also the N_2O-N emissions fall significantly down, in good agreement with previous experiences (Tsuneda et al., 2005). Nevertheless, at the end of the Phase II the AOB activity was partially recovered, leading to a reduction of the effluent ammonia as well as to a significant NO_2-N accumulation within the aerobic compartment (Mannina et al., 2016b): as a consequence, the N_2O production increased again. More precisely, referring to the experimental day 80, the N_2O-N concentration in the gas phase was respectively 30 and 76 times higher than that measured in the previous sampling day (Fig. 3a). Therefore, the shock caused by the joint effect of hydrocarbon and high salinity was partially overcome once being acclimated. However, the NOB activity remained severely affected, thus suggesting that longer durations might be necessary to restore the whole nitrification ability of the system.

Referring to the N_2O-N fluxes (Fig. 3b), 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 reflected the same pattern of the N_2O-N concentration from day 35 to day 75. It is worth noting that the flux emitted from the aerated tanks was 1–2 order of magnitude higher than that emitted from the anoxic one (Yang et al., 2009; Daelman et al., 2012; Law et al., 2012b).

Data reported in Fig. 3c refer to the 35th experimental day, when salinity and hydrocarbon concentration were 20 gNaCl L^{-1}

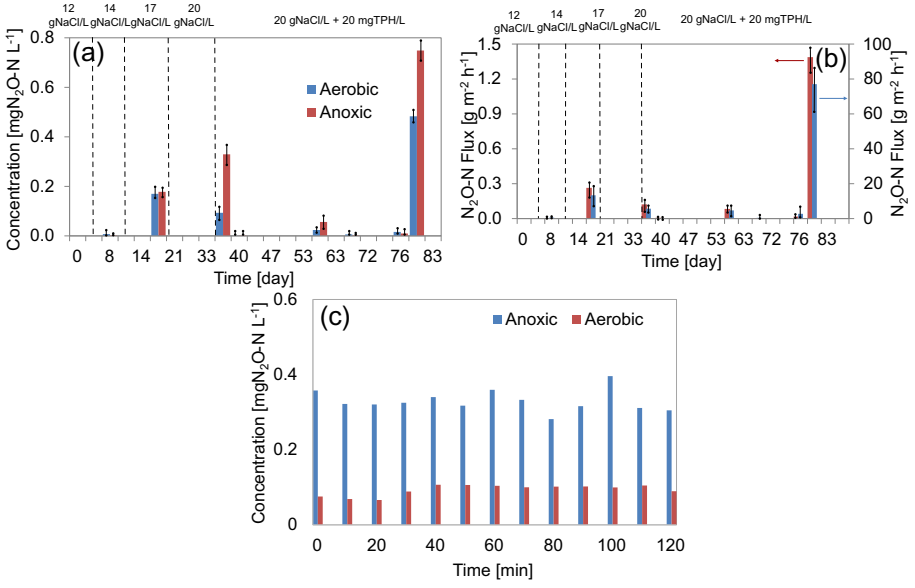

Fig. 3. Gaseous N_2O concentration (a) and N_2O flux (b) in aerobic and anoxic tank; ●—● standard deviation; N_2O-N concentration (c) at the 35th experimental day in aerobic and anoxic tank.

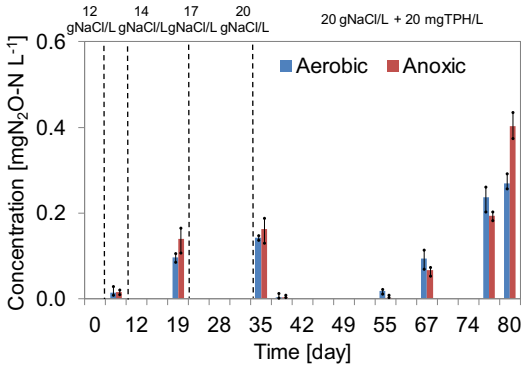

Fig. 4. Liquid N_2O concentration in aerobic and anoxic tank; ●——● standard deviation.

and 20 mgTPH L^{-1} respectively (Phase II). By analysing Fig. 3c it is possible to observe a slight predominance of N_2O production in the anoxic phase compared to the aerobic one; moreover, the nitrous oxide concentration was almost constant during the whole sampling period.

3.4. Dissolved N_2O-N concentration

Fig. 4 reports the results in terms of dissolved N_2O-N concentration both in aerobic and anoxic tank. By analysing Fig. 4, it is worth noting that the dissolved N_2O-N concentration had almost the same value in aerobic and anoxic tank. The average value measured during the sampling period was 0.11 mg N_2O-N L^{-1} (SD = 0.06 mg N_2O-N L^{-1}) in the aerobic tank and 0.13 mg N_2O-N L^{-1} (SD = 0.09 mg N_2O-N L^{-1}) in the anoxic one (Fig. 4). Nevertheless, it is important to notice that the N_2O-N concentration in the liquid phase confirmed the data related to the gaseous phase highlighting the significant influence of the salt and hydrocarbon inhibition effect. Indeed, the inhibition of AOB and NOB activity, occurring between days 35th and 65th, led to a negligible N_2O concentration dissolved in the mixed liquor.

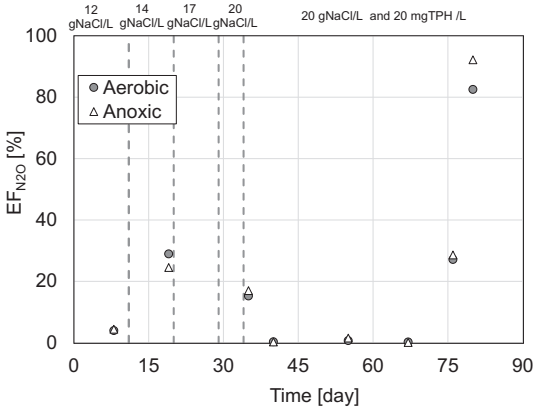

Fig. 6. N_2O emission factor pattern during experiments.

3.5. Factors affecting the N_2O-N production

3.5.1. Denitrification efficiency

As discussed above, one of the main effects of salt and hydrocarbon addition was the partial nitrification and denitrification. This result is in line with previous studies (Kampschreur et al., 2009) and led to different nitrogen pathways, thus promoting the N_2O production. Fig. 5a shows the N_2O-N concentration vs the denitrification efficiency. From Fig. 5a, it is possible to observe that, as soon as the denitrification efficiency is low, the N_2O-N concentration, that is an intermediate of the sequential reduction of NO_3-N to N_2 gas, is high. As matter of the fact, there is a good correlation between N_2O-N in gaseous samples 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 Fig. 5a. The outliers are the sampling related to the experimental day during which the biomass activity was severely

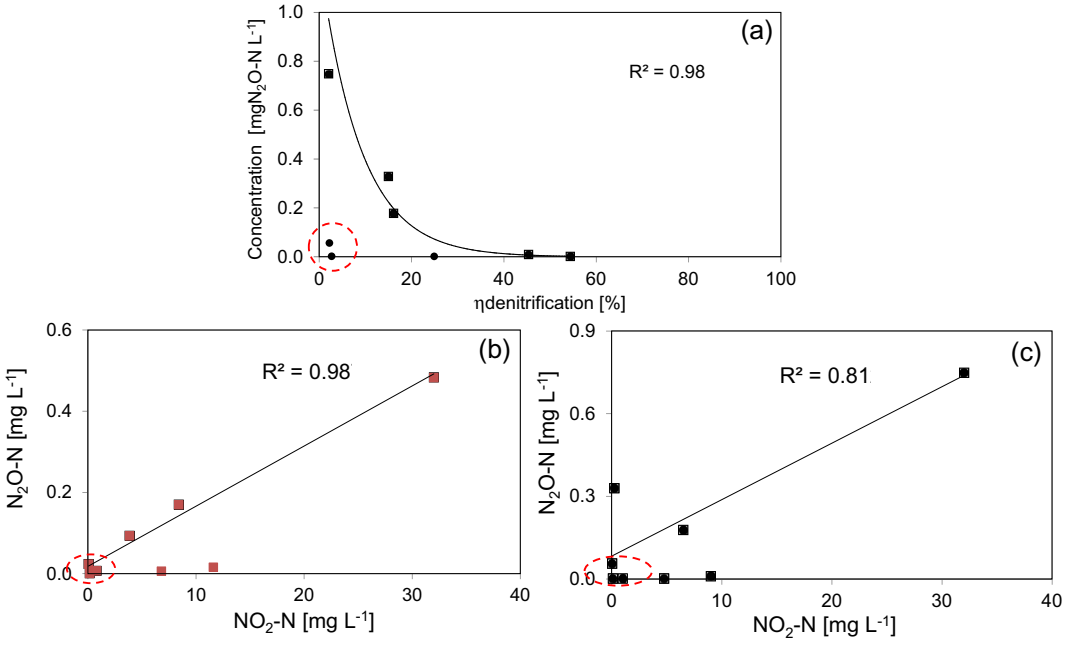

Fig. 5. Nitrous oxide and denitrification efficiency in the anoxic tank (a); N_2O-N concentration and nitrite in aerobic (b) and anoxic (c) tanks; the red circles highlight the point excluded by the correlation (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

inhibited by the joint shock effect of hydrocarbons and high salinity (Phase II at 20 g NaCl L^{-1} and 20 mg TPH L^{-1}).

3.5.2. Influence of nitrite accumulation on N_2O-N emission

Fig. 5(b and c) reports the relationship between nitrite and gaseous N_2O concentration in the aerobic (Fig. 5b) and anoxic (Fig. 5c) tank, respectively. By analysing Fig. 5(b and c), one can observe a good correlation between nitrite accumulation in the liquid phase and N_2O-N emission. It is important to specify that data related to the days of the complete inhibition of autotrophic bacteria (first 35 days at 20 gNaCl L^{-1} and 20 mgTPH L^{-1}) have been excluded from the correlation reported in Fig. 5(b and c) and are highlighted by the red circle.

The results reported in Fig. 5(b and c) show a linear dependency between the NO_2-N accumulation and the N_2O-N production both in the aerobic and anoxic tank. This behaviour corroborates 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; Kampschreur et al., 2009; Yang et al., 2009).

3.6. N_2O emission factors

Fig. 6 depicts the N_2O emission factors, expressed as a percentage of the nitrogen loading rate, for each feeding salt rate and experimental phases.

From the observation of Fig. 6, it is worth noting that the N_2O emission factor trend follows the nitrification activity. Indeed, as soon as the nitrification efficiency collapsed to values close to zero, no NO_2-N nor NO_3-N were produced (among others, Mannina et al., 2016b) and therefore the N_2O emission factors dropped down to zero. During the last days of the plant operation, when the nitrification was partially recovered, an increase of the emission factors occurred, reaching at the end of the experimental period values of 82% and 92% for the aerobic and anoxic tank, respectively.

Indeed, the observed emission factors at the end of experiments were slightly higher compared to literature data obtained from an oxic-anoxic activated sludge pilot plant subjected to a salinity increase (Tsuneda et al., 2005). The higher values achieved in the present study could be a consequence of the joint effect of salt and hydrocarbon that significantly disturbed the biological performance of the system, thus promoting the N_2O emission and causing as a consequence a potential harmful effect on the environment.

4. Conclusions

This study highlighted that the joint effect of salinity variation and hydrocarbons dosage may have a huge impact on N_2O production, due to the weakened biological performance (nitrification/denitrification) and thus potentially harmful for the environment. The joint effect due to hydrocarbons and salt concentration led to a temporary complete inhibition of the biological processes, stopping as a consequence the production of N_2O. Once the biological processes partially recover, the N_2O production can start again. As final remark, it is suggested that a gradual increase of salt and hydrocarbons and prolonged acclimation durations are required to enhance a reduction of N_2O production.

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