NO absorbents as mass transfer vectors for biological treatment

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Abstract

The treatment of nitrogen oxides (NOx) has been recently addressed using biological technologies. However, nitric oxide (NO) removal by biological technologies is quite challenging due to the low solubility of NO in water (0.00618 g NO·100 g^{-1} H_2O). The treatment of NO emissions through biological technologies requires a chelating agent or a mass transfer vector to increase the solubility of this pollutant into the aqueous phase where the bioprocess takes place. This research seeks to assess the performance of different non-aqueous phase solvents (NAPs) as mass transfer vectors and their suitability to be used as absorbents in a chemical absorption biological reduction (CABR) integrated system. The results showed that hexadecane and heptamethyl nonane were able to remove a maximum of 2.47E-04 moles NO / moles NAP and 2.61E-04 moles of NO / moles NAP, respectively. When an aqueous phase was added to the system, the absorption of NO was increased, with heptamethyl trisiloxane reaching an elimination of NO of 82 ± 3% with water and 88 ± 6% with a buffer solution. All NAPs were tested for short-term toxicity and resulted non-toxic. However, diethyl sebacate showed high biodegradability that would limit its applicability in biological processes for gas treatment.

1. Introduction

NOx gases are produced by human activities in the combustion of fossil fuels. The transport and industrial sectors, as well as power plants, are the main pollution sources from combustion of fossil fuels. In general terms 95 % of NO_x in combustion flue gases are NO, while the remaining 5 % are NO_2. These gases contribute to both eutrophication and acidification of ecosystems and can lead to negative health effects. Currently, the main physicochemical technologies being used for controlling NO_x emissions from combustion gases are selective non catalytic reduction (SNCR), selective catalytic reduction (SCR) [1], scrubbing and adsorption. However, these techniques have two main drawbacks: their high cost, because they use either expensive reagents or additional fuel as energy sources, and their high environmental impact, due to the large amounts of hazardous waste (secondary pollutants) they generate.

Therefore, the development of low-cost and sustainable alternatives for the treatment of NO_x emissions is required. In this sense, biological treatment is now being seen as an alternative to conventional treatment technologies. However, the biological treatment of NO emissions has only been performed successfully on a laboratory scale, due to the low solubility of NO [2]. One of the bio-based alternatives is the chemical absorption and biological reduction (CABR) process, which includes a previous stage to the biological reduction of NO were a chemical absorption or complexation step takes place through the use of either a mass transfer vector or a chelating agent [3]. The most widely used chelating agent to improve solubility has been $Fe(II)EDTA^{2-}$. However, it has many limitations such as its oxidation and system unstability. Therefore, the search for a new mass transfer vector is a challenge for the scientific community.

Different non-aqueous phase solvents (NAPs) have been studied for the absorption of gases. Hexadecane and silicone oil were studied to improve the mass transfer of volatile organic compounds (VOCs) and CO_2 [4], [5]. Diethyl sebacate, heptamethyl nonane, and heptamethyl trisiloxane were used as mass transfer vectors to improve absorption in VOCs such as alpha pinene, naphthalene and anthracene [6]. Within his research a detailed study is

performed to validate the viability of some NAPs as mass transfer vectors to improve the solubility of NO in water for further treatment with biological technologies (e.g. biotrickling filters, two-phase partitioning bioreactors, etc.).

2. Materials and methods

2.1. Chemicals and gases

NAPs (n-hexadecane (HEX), diethyl sebacate (DSE), 1,1,1,3,5,5,5-heptamethyl trisiloxane (HTX), 2,2,4,4,6,8,8-heptamethyl nonane (HNO) and high temperature silicone oil (SO)) were purchased at Sigma Aldrich (Lyon, France). All chemicals purchased were of the highest purity grade available. NO (20% in nitrogen) and nitrogen were purchased from Linde gas España (Rubí, Catalonia, Spain).

2.2. Organism and growth conditions

Denitrifying biomass used in this study was obtained from an 8 litres Sequential Batch Reactor (SBR) inoculated with biomass from the anoxic treatment of municipal wastewater treatment plant (WWTP) located in Manresa, Spain. After inoculation, the denitrifying bacteria were enriched for 2 months in the Sequential Batch Reactor (SBR) that was configured to develop 2 cycles of 12 hours. Each cycle had a time of 13 minutes of filling, 11 hours and 15 minutes of anoxic reaction, 30 minutes of settle and 2 minutes of withdraw. It also had a pH control system set at pH 8 with 1 M HCl addition. At steady state the reactor had a total NO_2^- load of 0.8 g/L, 1.2 g/L $C_2H_3NaO_2 \cdot 3H_{2O}$, 0.016 g/L KH_2PO_4, 0.041 g/L $CaCl_2$ and 1 mL micronutrients solution (0.15 g/L H_3BO_3, 0.03 g/L $CuCl_2 \cdot 2H_2O$, 0.18 g/L KI, 0.12 g/L $MnCl_2 \cdot 4H_2O$, 0.06 g/L $NaMoO_4 \cdot 2H_2O$, 0.12 g/L $ZnSO_4 \cdot 7H_2O$, 0.15 g/L $CoCl_2 \cdot 6H_2O$ and 10 g/L EDTA.$Na_2O_8 \cdot 2H_2O$).

2.3. Microbial community analysis

The biomass of the SBR was sampled at the steady state for microbial composition study. DNA extraction from the sample was performed using the DNeasy PowerSoil Pro Kit (Qiagen, German) according to its principles and instructions and stored at -70ºC. Two variable regions (V3, V4) of the 16S rRNA gene were amplified by Polymerase Chain Reaction (PCR) with custom designed fusion primers described by Torrell et al. (2021). Bobine serum albumin (BSA) was added to the PCR reaction to neutralize potential inhibitors. The PCR product, called amplicon or library, was visualized with a 2% agarose gel, purified with the NucleoSpin kit (Macherey-Nagel, Berlin, Germany) and quantified with an Agilent 2100 Bioanalyzer (Agilent Technologies, California, USA) and the Agilent High Sensitivity DNA kit (Agilent Technologies). Lastly, an equimolar mixture (60pM) of the samples in three pools was created to be sequenced in three runs. From the equimolar mixture of libraries, the Ion 520 & Ion 530 Kit-Chef (Life Technologies, Carlsbad, California, USA) and a xip 530 were used for the sequencing of each sample group. Sequencing was performed on LifeTechnologies GeneStudio S5 equipment, using 850 flows per run. The analysis was performed using QIIME (2-2020.8).

2.4. Denitrifying capacity - qPCR and Gene Expression

The same sample used to study the microbial community was used to study denitrification genes expression. RNA extraction was done with RNeasy PowerSoil Total RNA Kit (Qiagen, German) according to its principles and instructions and stored at -70ºC until analysis. From the set primers published by Razaviarani et al. (2019), three pairs of primers were synthesized to selectively amplify the nirS, CnorB and nosZ genes. RNA samples were retrotranscribed with a commercial polymerase (Super script IV (18090050)) and assayed by real-time quantitative PCR for all 3 genes, using a Thermofisher polymerase with sybrgreen and the Applied biosystems 7900HT quantitative PCR kit.

2.5. Experimental set up

2.5.1. Absorption tests

Batch tests with pure NAPs were performed in 120 ml glass amber vials with an initial concentration of NO between 4500 and 5100 ppm_v and different amounts of one of the tested NAPs (DSE, SO, HTX, HNO and HEX). Batch tests of NAPs in contact with an aqueous phase were carried out in 500 ml glass amber bottles. Tests were performed with water and phosphate buffer at pH 8 at different ratios of NAP/aqueous phase (5, 10, and 20 %v/v). Initial NO concentration between 4500 and 6000 ppm_v was used. Vials and bottles were previously prepared in a glove box to maintain an inert atmosphere. All experiments were carried out per triplicate at a temperature of 25 ºC and agitation of 100 rpm in an incubator.

NO and NO_2 concentrations in the gas phase were measured at the beginning and at the end of the test (1 hour) by Fourier Transform Infrared Spectroscopy (FTIR) (PerkinElmer Inc., Spain). The liquid phase was monitored for pH, nitrite (NO_2^-) and nitrate (NO_3^-) at the end of each test. Nitrite and nitrate concentrations were measured using Hach Lange kits (LCK342 and LCK 339, respectively, Hach Lange, Germany).

2.5.2. Toxicology and short-term biodegradation test with denitrifying bacteria

Toxicity and short-term biodegradability tests were performed using an AER-500 respirometer (Challenge technology®) with an anaerobic configuration. The tests were carried out in 500 mL glass bottles containing 300 mL of denitrifying bacteria from the SBR at 0.95 g VSS/L and 50 mL of NAP. The production of gas in the form of nitrogen was measured to monitor the biodegradability and/or toxicity of NAPs.

2.5.3. Long-term biodegradability tests.

The ultimate biochemical oxygen demand (BOD) tests (long-term biodegradability) were performed with OxiDirect® BOD measuring device and according to what is described in the Organization for Economic Co-operation and Development (OECD) guide for testing chemical products of respirometry manometric test (301F). Oxygen consumption due to pressure change over 28 days was measured. The tests were carried out in 500 mL glass bottles using 1 mL of biomass from the SBR at a final solid's concentration of 0.007 g TSS/L, 156 mL of mineral medium (composition can be found in the OECD guide), and an amount of NAP (175 mg HEX, 4169 mg HNO, 88 mg HTX, 66 mg DES or 66 mg SO).

2.5.4. Chemical absorption and biological reduction - CABR tests

CABR batch tests (four replicates per test) were performed in 120 ml glass amber vials previously filled with N_2. Experimental conditions were a temperature of 25 ºC and agitation of 100 rpm. Initial NO concentration between 4500 and 6000 ppm_v was used. Three experiments were performed varying the ratio between biomass and NAP. In the first experiment, 20 mL of biomass with a concentration of 1.09 g/L were introduced, followed by 2.5 mL of HTX, HNO or HEX in the same vial. In the second, the biomass concentration was increased to 1.88 g/L and each NAP was maintained at 2.5 mL. In a last experiment, a biomass concentration of 2.7 g/L and 1 mL of each NAP were used. All experiments had a blank containing neither NAP nor biomass and a control containing only biomass. The concentrations of NO and NO_2 in the gas phase were measured at the beginning and for 4 hours every 30 minutes. Also, the phase liquid was monitored for pH, NO^{2-} and NO^{3-} at the end of each test.

3. Results

3.1. Nitrogen oxide absorption with pure NAP's

Figure 1 shows the reduction of NO in the gas phase in the presence of pure SO, DSE, HTX, HNO, and HEX at different amounts of NAP. Increasing the amount of NAP produced a greater removal of NO in the gas phase and, therefore, the amount of NO removed depends on the amount of NAP available. A linear regression of the data in Figure 1 gave a slope that indicates the NO removal capacity of each NAP. The results showed that HTX, HEX and HNO were able to remove a maximum of 2.32E-04 moles of NO / moles of NAP, 2.47E-04 moles of NO / moles of NAP and 2.61E-04 moles of NO / moles of NAP, respectively.

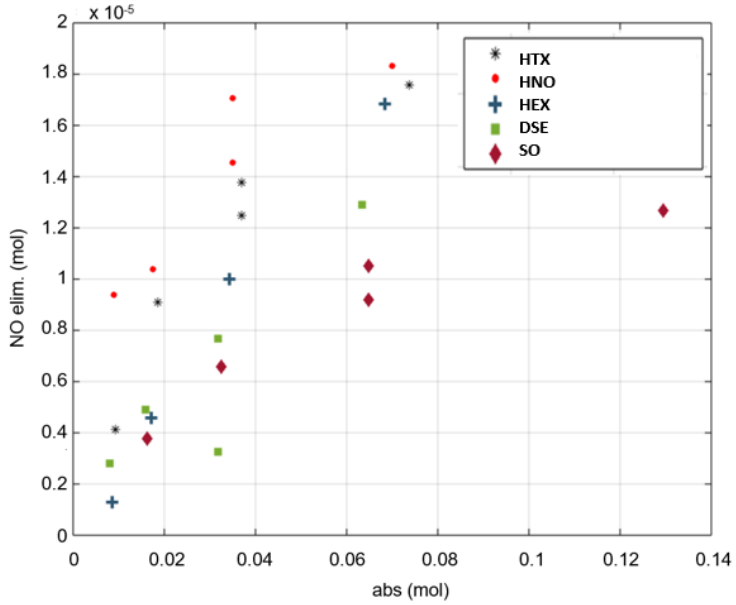


Figure 1 Amount of NO removed from the gas phase vs amount of pure NAP as absorbent.

3.2. Nitrogen oxide absorption in aqueous phase with NAPs

Figure 2 shows the removal efficiency of NO using (HEX, HNO, HTX, SO, DSE) in aqueous phase (water) And Figures 3 shows the removal efficiency of NO using (HEX, HNO, HTX, SO, DSE) in aqueous phase (phosphate buffer (pH 8). The results indicate that when the percentage of the liquid phase decreases, the yield increases because there is more liquid phase in the medium that retains the nitrite. Additionally, the absorption of NO was observed to be higher than the absorption of the pure NAPs (white bar in Figures 2 and 3). The NAPs that gave the best results in terms of percentage of removed NO in the gas phase when in contact with the aqueous phase were DSE (72 ± 4% in water; 71± 6% in buffer), HTX (82 ±3% in water; 88±6% in buffer) and HNO (40 ± 2% in water; 47±3% in buffer). Furthermore, no significant difference in the percentage of absorption was observed if buffer or water was added. Also, a drop in pH from about 6 to about 3 was observed when using water. However, in the tests with phosphate buffer, the pH was kept at 8 even though NO tends to acidify the medium.

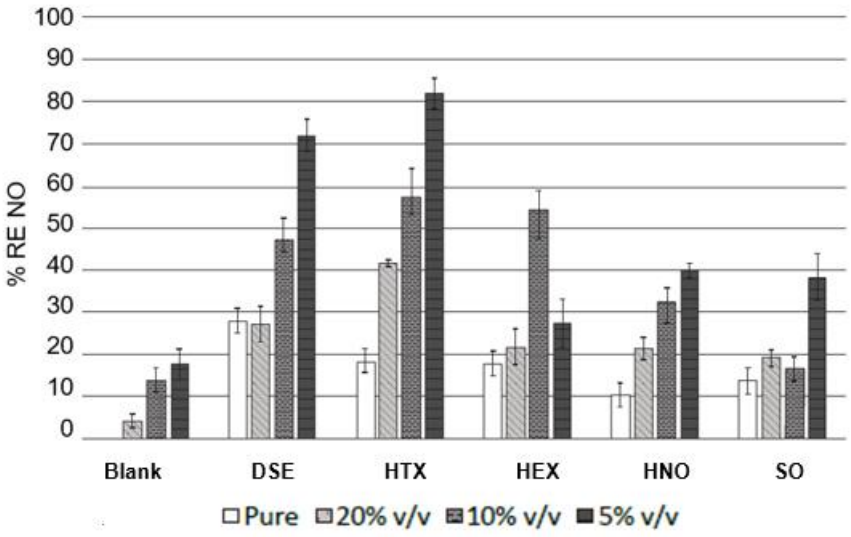


Figure 2 NO removal efficiency (RE) percentage using the different NAPs in aqueous phase (water). Error bars indicate the standard deviation of three replicates.

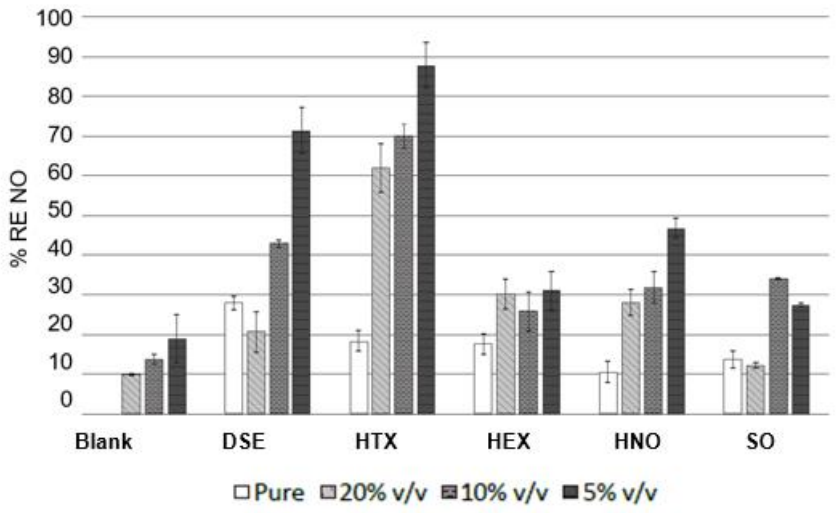


Figure 3 NO removal efficiency (RE) percentage using the different NAPs aqueous phase (buffer). Error bars indicate the standard deviation of three replicates.

3.3. Toxicity and biodegradation test with denitrifying bacteria

None of the NAPs studied were found to be toxic for denitrifying bacteria with short-term tests. All except DSE had no apparent biodegradability under these conditions. Short-term biodegradability tests showed that all NAP except for DSE were nonbiodegradable. Long-term tests showed a maximum of 8 % biodegradation. Specifically, 0 % for SO, 1 % for HNO, 7% for HTX and 8 % for HEX. The test also confirmed that DSE was completely biodegradable under anoxic and aerobic conditions. As the toxicity and biodegradability of these compounds with denitrifying bacteria have not been previously studied, these results will serve as a guide for future applications in the industry.

3.4. Chemical absorption and biological reduction - CABR tests

With the results of the abiotic and biotic tests, three absorbents (HEX, HNO and HTX) were chosen to study the integration of the chemical absorption and biological NO reduction system. Figure 4 shows the removal efficiency of NO with biomass with a concentration of 1.09 g/L (1) – 1.88 g/L (2) and 2.5 mL of blank, NRB or NAP. NO elimination was shown to improve in the short term (30 min or 1 hour) when the three mass transfer vectors and enriched denitrifying bacteria were used. It can also be seen that by increasing the biomass concentration from 1.09 g/L (test 1) to 1.88 g/L (test 2), NO removal increased. For example, NRB (Nitrate/Nitrite bacteria) - HTX reached 100% NO removal at three hours with a biomass concentration of 1.88 g/L, whereas with 1.09 g/L it reached only 72% of removal efficiency (RE) NO.

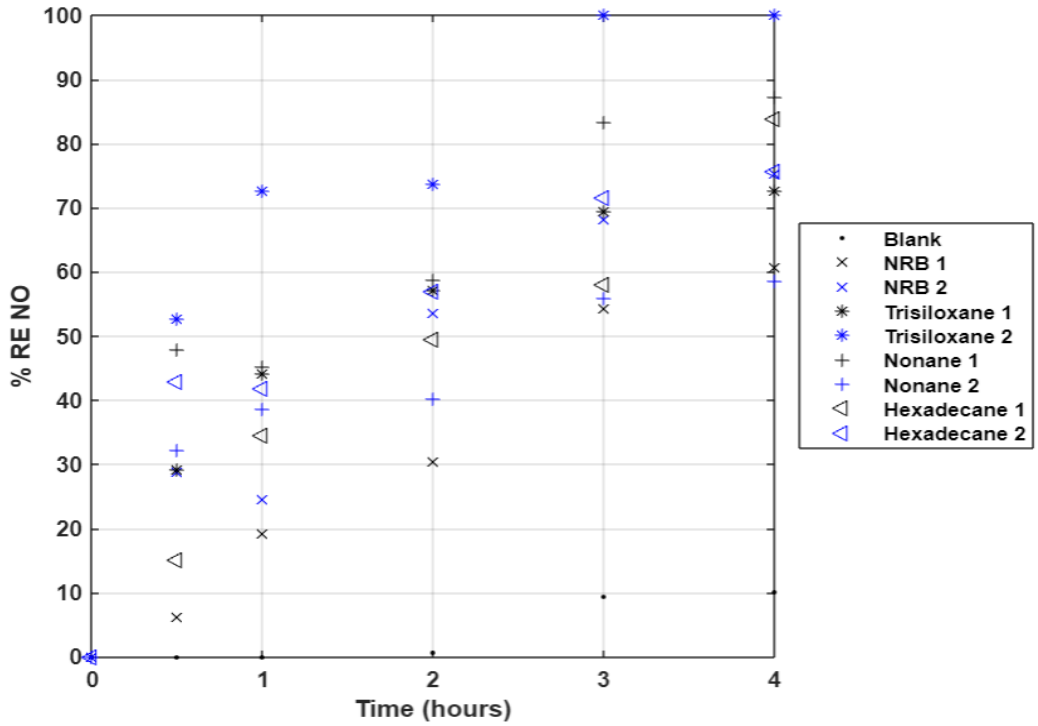


Figure 4 Removal efficiency percentage of NO with biomass with a concentration of 1.09 g/L VSS (test 1) – 1.88 g/L VSS(test 2) and 2.5 mL of NAP

Additionally, in the Figure 5 andat 1 and 24hours Figure 6 it is observed that when biomass is present, the accumulated nitrate decreases over time. In contrast, nitrite generally increases with time; but in both cases, the accumulation is lower than in the experiment without biomass and therefore, this suggests that the nitrogenous compounds are reduced to N_2 gas by the action of the microorganisms. The same non-accumulation in the system was evidenced for HEX and HNO.

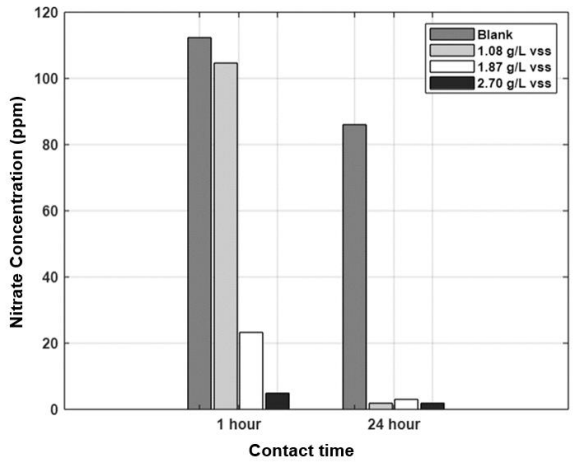


Figure 5 Concentration of nitrate of HTX in the three experiments at 1 and 24hours

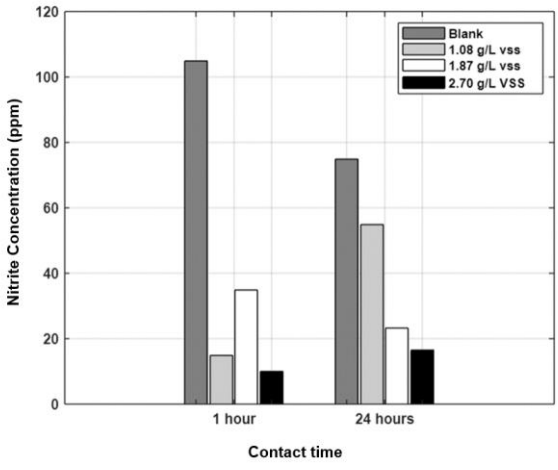
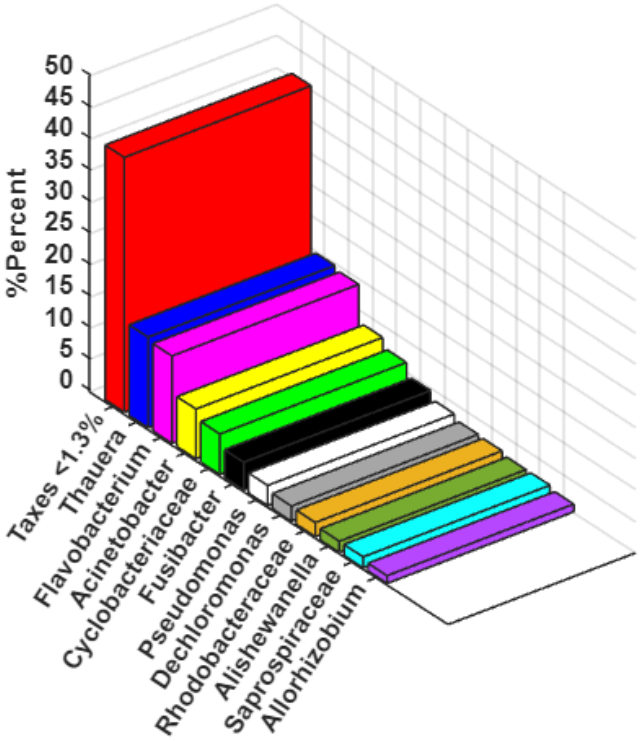


Figure 6 Concentration of nitrite in the three experiments of HTX at 1 and 24 hours

3.5. Microbial community analysis

The microbial community structure was analysed by next generation sequencing to investigate the species existing in the biomass used for the CABR test. As shown in the Figure 8, at order level, *Thauera* (14.37%), *Flavobacterium* (13.87%), *Acinetobacter* (7.90%), *Cyclobacteriacea* (6.41%), *Fusibacter* (4.41%), *Pseudomonas* (3.19%), *Dechloromonas* (2.40%), *Rhodobacteraceae* (2.25%), *Alishewanella* (1.75%) and *Saprospiraceae* (1.74%) were

dominant. From these species, Thauera, Flavobacterium, Acinetobacter, Pseudomonas and Rhodobacteraceae are known to be denitrifying bacteria, thus the biomass used in these experiments contained, as expected, mainly denitrifying bacteria.



3.6. Denitrifying capacity: Gene Detection and Gene Expression by qPCR

Table 1 shows the presence or absence of denitrifying genes was assessed by checking if these genes appear in the completed genomes of all the taxonomic categories that were found by the metagenomic approach. It can be seen that the most common gene found was nosZ, being *Thauera*, *Flavobacterium*, *Pannonibacter* the genera that were most abundant in the analyzed sample and were carrying this gene. Presence of all three genes (nosZ, nirS and CnorB) was only found in Pseudomonas. Additionally, the genera *Streptococcus*, *Bacillus*, *Legionella*, *Streptomyces* and *Corynebacterium* were found to have the presence of the CnorB gene.

Table 1 Presence/absence of nosZ, nirS and CnorB the genome of the identified genes. Percentage in abundance reflects the contribution of this genera to the overall sample of the biomass used in the experiments

Identified Genus	**Percentage of abundance in the sample**	**Presence of the gene in the genome**		
		nosZ	**nirS**	**CnorB**
Thauera	13.95%	Present	Absent	Absent
Pannonibacter	4.69%	Present	Absent	Absent
Flavobacterium	4.52%	Present	Absent	Absent
Chryseobacterium	0.80%	Present	Absent	Absent
Pseudomonas	0.41%	Present	Present	Present
Rhodobacter	0.29%	Present	Absent	Absent
Hyphomicrobium	0.20%	Present	Absent	Absent
Paracoccus	0.17%	Present	Absent	Absent
Shinella	0.14%	Present	Absent	Absent
Mesorhizobium	0.09%	Present	Absent	Absent

Identified Genus	Percentage of abundance in the sample	Presence of the gene in the genome		
		nosZ	nirS	CnorB
Bacteroides	0.06%	Present	Absent	Absent
Bacillus	0.05%	Present	Absent	Present
Streptomyces	0.04%	Absent	Absent	Present
Prevotella	0.03%	Present	Absent	Absent
Bradyrhizobium	0.03%	Present	Absent	Absent
Legionella	0.03%	Absent	Absent	Present
Streptococcus	0.02%	Absent	Absent	Present
Castellaniella	0.02%	Present	Absent	Absent
Ensifer	0.01%	Present	Absent	Absent
Afipia	0.01%	Present	Absent	Absent
Elizabethkingia	0.01%	Present	Absent	Absent
Corynebacterium	0.01%	Absent	Absent	Present
Luteitalea	0.01%	Present	Absent	Absent

Additionally, the genetic expression of all three genes were anaylzed by qPCR (using specific primers and sybrgreen). Only nosZ could be detected, as it is the most present in the bacterial community. nirS and CnorB are assumed to be or non-expressed or expressed under limit of detection.

4. Discussion

This study establishes the applicability of the use of different NAPs in CABR technology from the aspect of the chemical absorption process and biological reduction, giving the basis for new research in the biological treatment of NO.

Only organic tertiary hydroperoxides in HEX solution were studied as oxidants to improve NO solubility [9]. The rest of the NAPs that were tested in this study were not previously studied with NO. However, they can be an innovative option to test them with NO, since they have been investigated for other gases, even though they have different physical and chemical properties. For instance, Yeom and Dauguris (2000) studied in a bubble column the use of HEX as an absorbent for benzene achieving 90% removal with an inlet benzene concentration of 8.5 mg/l and a gas flow rate of 120 l/h.

Regarding the non-toxicity of the NAPs, other studies with different denitrifying bacteria found that the NAPs studied do not present toxicity. For instance, Köhler et al. (1994) studied the degradation of phenanthrene by *Pseudomonas aeroginosa* AK1 (denitrifying bacteria) using HNO as a mass transfer vector (MTV), confirming that HNO improves the solubility of anthracene without being toxic to AK1. Also, Marcoux et al. (2000) studied the degradation of high-molecular-weight polycyclic aromatic hydrocarbons (HMW PAHs) such as pyrene, chrysene, benzo[a]pyrene and perylene with SO, HNO and HEX as MTV and using the microorganism Pseudomonas aeruginosa 57RP, verifying that the denitrifying bacteria did not present toxicity in the presence of these NAPs.

With regard to the biodegradability of the NAPs tested, Muñoz et al. (2008) also showed that SO, HEX, HNO and HTX are biocompatible and non-biodegradable with bacteria, specifically with the denitrifying bacterium *Pseudomonas fluorescens* NCIMB 11671. Furthermore, the results of their study support the results of this study regarding the fast mineralization of DSE in the presence of denitrifying microorganisms.

The analysis of the bacterial diversity carried out in this investigation found that the dominant species in the system were denitrifying bacteria. Normally denitrification occurs under low oxygen and anoxic conditions. However, recent studies have found that some bacteria present in this study can tolerate the presence of oxygen without being

affected by the denitrification process. For instance, *Thauera, Flavobacterium and Rhodobacteraceae* were reported as aerobic denitrifying bacteria and cannot be inhibited by oxygen in a CABR system using $Fe(II)EDTA^{2-}$ [14-15]. Also, typical denitrifying bacteria were found in our system. For example, The genus *Pseudomonas* and *Acinetobacter* includes the most commonly isolated denitrifying in WWTP [16].

Looking for the published sequence of the genera that were detected through the study of bacterial diversity, it was concluded that the 3 selected genes were present in the sample, nosZ being the one found in more different species and the one found in greater relative proportion. Additionally, the gene expression study performed by qPCR showed a clear expression of the nosZ gene, while the expression of nirS and CnorB was not positive. As a conclusion, it can be assumed that the denitrifying capacity is mainly due to nosZ, rather than to the other 3 genes. It must be said that even if a gene is present in the genome, it does not mean that it is constantly expressing it. That is, the presence of the gene does not indicate its expression because the expression of a gene occurs at a given time or under a specific stimulus.

5. Conclusions

The results of this work showed that all the NAPs (HEX, HNO, HTX, DSE, SO) could be used as mass transfer vectors for chemical absorption - biological reduction systems of NO, with heptamethyl trisiloxane being the compound that showed the greatest absorption capacity both in its pure state and in contact with an aqueous phase. Additionally, none of the NAPs studied turned out to be toxic for denitrifying bacteria. However, diethyl sebacate resulted to be biodegradable, which, to be used in biological processes, would generate high operational costs because NAP could not be reused in the process. In the CABR tests, it was shown that NO elimination improves in a short time (30 minutes) when the three mass transfer vectors (HEX, HTX, HNO) and enriched denitrifying bacteria are added.

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