Mónica Luís Pereira Rodrigues

Licenciatura em Ciências de Engenharia do Ambiente

Dissolved Organic Nitrogen behaviour during denitrification with suspended and attached biomass

Dissertação para obtenção do Grau de Mestre em
Engenharia do Ambiente, perfil Engenharia Sanitária

Orientador: Professora Doutora Leonor Miranda Monteiro do Amaral,
Professora Auxiliar FCT/UNL
Co-orientador: Engª Diana Teixeira D'Aguiar Norte Brandão, TU Delft

Jurí:
Presidente e Arguente: Prof. Doutor António Pedro de Macedo Coimbra Mano
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FACULDADE DE CIÊNCIAS E TECNOLOGIA
UNIVERSIDADE NOVA DE LISBOA

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“It is not enough to be busy.

So are the ants.

The question is: What are we busy about?”

Henry David Thoreau

[1817-1862]

Resumo

Numa estação de tratamento de água residual o azoto orgânico dissolvido (DON) representa uma fracção importante de N no efluente final. Este trabalho tem por objectivo a análise do DON ao longo do processo de desnitrificação, com biomassa suspensa e fixa.

Para tal, foram feitos ensaios experimentais de desnitrificação com lamas activadas e suportes (*kaldnes*) de filme biológico. Para os ensaios com *kaldnes* foram utilizadas duas velocidades de mistura diferentes para a avaliação da sua influência nas concentrações de DON. Em todos os ensaios as concentrações de COD e DOC foram avaliadas. As concentrações de DON no efluente original e durante os processos de desnitrificação com *kaldnes* foram comparadas.

As concentrações de DON variam entre 0.1 e 1.9 mg/l durante os processos de desnitrificação. A comparação feita entre as concentrações de DON no efluente original e as medições de DON durante os ensaios confirma que DON tem uma percentagem significativa (aproximadamente 20%) de N no efluente final. Os resultados obtidos indicam que DON tem uma variação constante ao longo do processo de desnitrificação.

Palavras-chave: Água residual, azoto orgânico dissolvido, desnitrificação, lamas activadas, suportes *kaldnes*.

Abstract

In a wastewater treatment plant (WWTP) dissolved organic nitrogen (DON) represents an important fraction of N in the final effluent. The purpose of this study was to analyze DON changes along denitrification with suspended and attached biomass.

Denitrification batch experiments were carried out with activated sludge and biological (*kaldnes*) carriers. In the batch tests with *kaldnes* it was used two different mixing velocities to evaluate its influence in DON concentrations. For the batch tests were evaluated COD and DOC concentrations. A comparison of the fate of DON during a denitrification process and the original effluent DON concentration was made.

DON determinations oscillate in a range from 0.1 to 1.9 mg/l during a denitrification test. The comparison between DON determinations and the original effluent confirm that DON has a significant portion (about 20%) of the effluent N. The results obtained indicate that DON has a constant behavior along the denitrification process.

Key words: wastewater, dissolved organic nitrogen, denitrification, activated sludge, *kaldnes* carriers

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Abbreviators

BNR	Biological nutrient removal
$C_2H_9NaO_5$	Sodium Acetate Trihydrate
CO_2	Carbon dioxide
COD	Chemical oxygen demand
DCAA	Dissolved combined amino acids
DFAA	Dissolved free amino acids
DHAA	Dihaloacetic acids
DIN	Dissolved inorganic nitrogen
DO	Dissolved oxygen
DOC	Dissolved organic carbon
DOM	Dissolved organic matter
DON	Dissolved organic nitrogen
EPS	Extracellular polymeric substances
H_2O_2	Hydrogen peroxide
H_2SO_4	Sulfuric acid
HNP	Harnaschpolder
HSO_4^-	Hydrogen sulphate
K_2HPO_4	Dipotassium phosphate
K_2SO_4	Potassium sulfate
N	Nitrogen
N_2	Nitrogen gas
Na_2HPO_4	Disodium phosphate
$NaH_2PO_4{\cdot}2H_2O$	Sodium dihydrogen phosphate
$NaNO_3$	Sodium nitrate
NDMA	N-nitrosodimethylamine
NH_3	Ammonia
NH_4^+	Ammonium
NO_2	Nitrite
NO_3^-	Nitrate
NOM	Natural organic matter

$S_2O_8^{2-}$	Peroxodisulfate ion
SAT	Solution of sodium acetate trihydrate
SDNR	Specific denitrification rates
TDN	Total dissolved nitrogen
THM	Trihalomethanes
TiO_2	Titanium dioxide
TKN	Total Kjeldahl nitrogen
TN	Total nitrogen
TOC	Total organic carbon
TSS	Total suspended solids
VSS	Volatile suspended solids
WWTP	Wastewater treatment plant

1. Introduction

1.1. General context

As an element that occurs naturally, nitrogen is a limiting nutrient for growth and reproduction of living organisms (Pagilla *et. al.*, 2011). Causing an increase in worldfood production and a decrease in hunger, nitrogen fertilizer has been an essential component of the Green Revolution (Howarth, 2004).

The human activities are changing nitrogen natural cycle, increasing the levels of total dissolved nitrogen in many surface waters through wastewater discharges, agricultural runoff, and NO_x deposition (Vitousek *et. al.*, 1997).

Although nitrogen is essential for living organisms, in extreme concentrations, often in nitrate form, presents a problem of growing concern for water-quality (Burgin and Hamilton, 2007). In excessive concentrations it holds severe threats to human health and to ecological functioning of both terrestrial and aquatic ecosystems, especially coastal marine ecosystems (Howarth, 2004).

Nitrogen pollution has a direct consequence in acidifying soils and waters, leading coastal marine ecosystems to eutrophication and loss of biodiversity in a variety of terrestrial and aquatic ecosystems (Howarth, 2004). Nowadays, eutrophication and other adverse effects are growing problems in many identified regions (Arnaldos and Pagilla, 2010).

Effluent discharged from municipal wastewaters and the stormwater runoff from urbanized areas, are an important antropogenic nitrogen source (Pehlivanoglu-Mantas and Sedlak, 2006). Due to the concerns related with the adverse effects of nitrogen, the installation of nitrification/denitrification systems at numerous municipal wastewater treatment plants (WWTP) was conducted (Pehlivanoglu-Mantas and Sedlak, 2004).

With the intent to protect the receiving water many municipal wastewater treatment facilities are facing the challenge of eliminating nitrogen and phosphorus to much lower effluent concentrations so that the eutrophication problem in surface waters decreases. To achieve these low values of nitrogen removal, biological nutrient removal (BNR) processes are pushed to their limits in order to transform ammonium, nitrate and nitrite (WERF, 2008).

The Water Framework Directive (2000), in the European Union, and the Environmental Protection Agency, in the United States have the intent to achieve very low nutrient levels, basically a discharge of total nitrogen (TN) below 3 mg/l (Arnaldos and Pagilla, 2010).

The majority of nitrogen discharged by WWTP is identified in most systems as being nitrate and ammonia. Dissolved organic nitrogen (DON), another important form of nitrogen, typically accounts for less than 10% of the nitrogen in the wastewater effluent (Pehlivanoglu-Mantas and Sedlak, 2004). However, when total TN has to be reduced to very low levels effluent DON becomes more important (Pagilla *et. al.*, 2006). Then with the successful removal of inorganic nitrogen DON becomes the majority fraction in the final effluents (from 56 to 95% of TON in secondary effluents) (Sattayatewa *et. al.*, 2009).

DON, in a practical sense, can be defined as that portion of organic nitrogen that passes through 0.45 micrometer (μ) membrane filters (Randtke *et. al.*, 1978).

Finally, DON is present in the total dissolved nitrogen (TDN) together with the inorganic species. Where TDN is (Lee and Werterhoff, 2005):

$$TDN = NO_3^- + NO_2^- + NH_3^+/NH_4^+ + DON$$

With more stringent effluent limits for TN, the concern of WWTP for the DON portion of the effluent nitrogen is increasing. Since DON is a precursor of potent carcinogenic nitrogenous disinfection byproducts (which are secondary products originated from drinking or waste water disinfection and may pose health risks (US EPA, 2013)), such as nitrosamines, nitromethanes and N-nitrosodimethylamine (NDMA), has also led to emerging concerns over the discharge of DON in effluents from wastewater or agricultural sources (Bratby *et. al.*, 2008).

Considering biological process effluents, the present dissolved organic matter (DOM) composed of soluble organic carbon and nitrogen may have microbial origin rather than the original organic substrates (Pagilla *et. al.*, 2008). Another concern is the shear force in a stable structure of biofilm, in a BNR process. The shear force (detachment force resulting from liquid flow and particle–particle collision (Liu and Tay, 2002)) leads to extracellular polymeric substances (EPS) production and its accumulation through biological shear stress. In denitrification processes the shear force can cause impact, changing its efficiency (Celmer *et. al.*, 2008).

From a wastewater treatment perspective it is essential to understand the transformations and fate of DON through a BNR treatment. The study of DON in a denitrification process is important to provide knowledge in the effluent quality, while aiming to achieve effluent limits for discharge.

1.2. Quality standards for wastewater discharge

Nutrient targets are largely a means to an end to manage water quality or achieve conservation objectives for a particular site. Therefore, with the European Water Framework Directive for the effluent there will be a change in, among others, nitrogen concentrations (Boelee, 2010).

Table 1.1 – Regulations for Total Nitrogen and future limits. (Adapted from Boelee, 2010)

	Current	Applicable from 2015 upfront
N (mg/l)	10	2.2

The current regulations for total nitrogen in wastewater treatment plant effluents are also in the United States approaching 5 mg/l or less to control eutrophication and hypoxia conditions in estuaries and bays. Though, with the current development in removal techniques, is possible to achieve a high inorganic removal, and consequently DON presence is maintained being the major nitrogen form (>50%) of the TDN effluent. So the challenge is now constituted by the residual DON, which makes part of about one-third to one-half of the effluent TN. To respect the discharge limits and meet a very low N, water quality criteria may be challenging and not economically sustainable, unless there is the development of new methods to address the fate and biodegradability of effluent DON (Simsek *et. al.*, 2013).

Thus, with the goals of lower TN effluent concentrations, in Europe and in the United States, the contribution of effluent organic nitrogen has currently gaining an increased importance.

2. Thesis Objective

The purpose of this research is to evaluate the variation of DON concentration during the process of denitrification of suspended and attached biomass. In order to achieve such purpose, denitrification batch experiments were carried out with activated sludge and biological carriers. A second objective was to evaluate the effect of shear stress on DON variation, for which different mixing velocities were used during denitrification batch tests with attached biomass. In both tests, the denitrification process was characterized through inorganic and organic nitrogen measurements, as well as COD, DOC, pH and temperature.

3. Literature Review

3.1. Nitrogen cycle

Nitrogen gas (N_2) is part of 78% of Earth's atmosphere and is a key element for proteins and cells (US EPA, 2010). It is the fourth most common chemical in living tissues, behind oxygen, carbon and hydrogen (Vitousek, 1997). The nitrogen that is present as molecular N_2 in the atmosphere and dissolved in the world's oceans, which is the majority of N on earth, only becomes reactive and biologically available to plants and algae through the process of bacterial nitrogen fixation, fixation by lightning and volcanic activity, and fixation from industrial activities including the manufacturing of inorganic nitrogen fertilizer and the combustion of fossil fuel (Howarth, 2004).

Thus, plants (and all organisms) must wait for the nitrogen to be "fixed", that is, getting to bond from the air with hydrogen or oxygen to from inorganic compounds, mainly ammonia (NH_3) and nitrate (NO_3^-), that they can use (Vitousek, 1997).

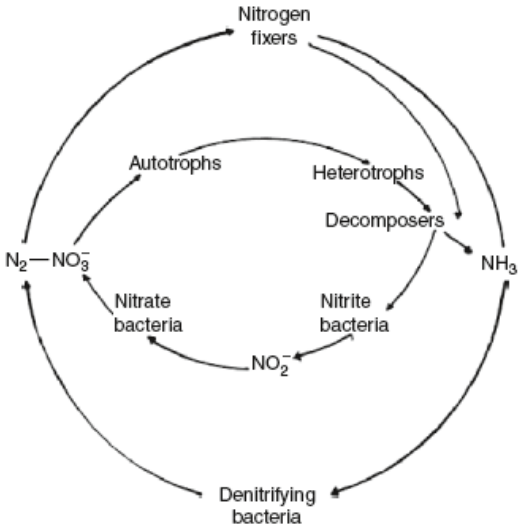

Figure 3.1 – Nitrogen cycle.

(Adapted from Bungay and Bungay, 2009)

The major transformations of nitrogen are nitrogen fixation, nitrification, denitrification, anammox, and ammonification and the transformation of nitrogen into its many oxidation states is key to productivity in the biosphere (Vitousek *et. al.*, 2002).

The biological conversion of ammonia/ammonium to nitrate is called nitrification. Nitrification is a two-step process. The first part up to nitrite (NO_2^-) is conducted by NH_3^-

oxidizers or primary nitrifiers, whereas the second step is carried out by NO_2^--oxidizers or secondary nitrifiers (Bock *et. al.*, 1986). The reactions are generally coupled and proceed rapidly to the nitrate form; therefore, NO_2^- levels at any given time are usually low. These bacteria only perform their work strictly in the presence of free dissolved oxygen (DO). Nitrification occurs only under aerobic conditions at DO levels of 1.0 mg/l or higher (The water planet company, 2013).

Denitrification is the biological reduction of oxidized nitrogen compounds like nitrate or nitrite to gaseous nitrogen compounds and it can be assimilatory and/or dissimilatory (Cortez *et. al.*, 2010). Assimilatory denitrification involves the reduction of nitrate or nitrite to NH_4-N for use in biomass synthesis when NH_4-N is not otherwise available. Most references to biological denitrification for nitrogen removal refer to dissimilatory denitrification in which nitrate/nitrite is the ultimate electron acceptor in the bacteria cell respiratory electron transport chain for the oxidation of various organic and inorganic substrates (US EPA, 2010).

The reactions are carried out by denitrifiers, using N_2O as an intermediate of denitrification which can be release in high quantities, when low-oxygen environments with sufficient NO_3^- and metabolizable organic carbon are present conditions. These predominantly heterotrophic microorganisms are facultative anaerobes that are able to use NO_3^- in place of oxygen as an electron acceptor in respiration to handle with low-oxygen or anaerobic conditions (Wrage *et. al.*, 2001).

The denitrification process involves the transfer of electrons from electron donor (i.e., carbon substrate as acetate) to the electron acceptor (i.e., oxygen, NO_2^- or NO_3^-); and the main factors affecting denitrification processes are identified as: the nature and amount of organic matter, nitrate concentration, aeration status (presence of dissolved oxygen), pH and temperature (Lin *et. al.*, 2009).

3.2. Nitrogen in wastewater treatment

As seen before, in **section 1.1**, nitrogen is an essential element but in excess poses severe threats to human health and to the ecological functioning, pointing out its principal contribution to eutrophication (Howarth, 2004). The negative impacts of eutrophication, as algae and phytoplankton growth which lead to harmful algal blooms, hypoxia, and loss of submerged aquatic vegetation, is one of the best documented and understood consequences of human alterations of the nitrogen cycle (Vitousek, 1997).

Eutrophication may also cause risks to human health, as from consumption of shellfish contaminated with algal toxins or direct exposure to waterborne toxins. Particularly, it can create problems in drinking water sources. As well excess levels of nitrates, above the maximum contaminant level, in drinking water can cause numerous negative health effects due to the body's conversion of nitrate to nitrite (US EPA, 2010).

Due to these reasons is important to limit nitrogen contamination. One way to minimize this impact is to reduce nitrogen levels in WWTP effluent. Thus, the elimination of nitrogen compounds is a fundamental aspect of wastewater treatment.

To eliminate nitrogen compounds, are applied BNR processes which are designed to oxidize NH_3-N to NO_3-N and/or NO_2-N, and reduce these compounds to N_2 by biological denitrification (WERF, 2008). Hence, nitrogen removal occurs in two sequential processes: nitrification and denitrification (US EPA, 2009).

A wastewater treatment contains one or more of the following processes: preliminary, primary, secondary, and tertiary treatment. The removal of grit, which removes dense inert particles and screening to remove tattered clothing and other large debris, is the preliminary treatment. Primary treatment entails gravity settling tanks to remove settleable solids, including settleable organic solids. Secondary treatment follows primary treatment in most WWTP and employs biological processes to remove colloidal and soluble organic matter (US EPA, 2009).

BNR can be accomplished by a variety of treatment configurations using suspended growth, attached growth, or combined systems (US EPA, 2009).

Currently, activated sludge is the most widely used treatment in BNR. Activated sludge consists of biological flocs that are matrices of microorganisms, nonliving organic matter, and inorganic materials (Wang *et. al.*, 2009). It is a biological process that utilizes microorganisms to convert organic and certain inorganic matter from wastewater into cell mass, oxidizing the organic substances in the presence of oxygen for bio-oxidation and nitrification reactions, or in the absence of oxygen for denitrification reaction (Marx *et. al.*, 2010).

A conventional activated sludge treatment for nitrogen removal includes several phases with different oxygen concentrations, first there is an aerobic reactor (where nitrification occurs) followed by a anoxic reactor (where denitrification occurs), or may employ only one reactor in which alternating aerobic and anoxic phases are achieved in time or space (Wang *et. al.*, 2009).

In conventional WWTP nitrogen removal is mostly achieved with pre-denitrification (Vocks *et. al.*, 2005). BNR can also be achieved by post-denitrification treatment such as denitrification filters (US EPA, 2010). To attain the intended low TN concentrations, in post-denitrification units and external carbon source is always added (Corona *et. al.*, 2013). Since without it the denitrification rate is expected to be low which demands an increase reactor volume to obtain a complete N-removal (Vocks *et. al.*, 2005).

To enhance nitrification and denitrification a suspended carrier technology is currently used (Lin *et. al.*, 2009). Among several other processes, the moving bed biofilm process is becoming increasingly popular (Ødegaard *et. al.*, 2000). It was developed adopting the best from both activated sludge process and the biofilter processes (Ødegaard, 1999). This process uses freely floating carriers that can be made of different materials, shapes and sizes (Lin *et. al.*, 2009). These carriers provide surface area for bacteria attachment that grows into a biofilm (Chu and Wang, 2011). During the moving bed biofilm reactor operation, the carriers are kept in constant circulation (Weiss *et. al.*, 2005). Contrary to most biofilm reactors the whole reactor volume is active, as does the activated sludge reactor (Ødegaard, 1999).

Nitrification and denitrification processes in a WWTP can remove 80 to 95% of inorganic N, but the removal of organic nitrogen is typically much less efficient. Organic nitrogen may even be released in secondary treatment by microorganisms either through metabolism or upon death and lysis (US EPA, 2009).

This research is going to focus in this aspect: the fate of DON concentration during the process of denitrification.

3.3. DON – Definition and Characterization

In a general description DON is that subset of the dissolved organic matter (DOM) pool that contains N. Comparing to dissolved organic carbon (DOC) research into DON has lagged far behind of the larger pool of DOC. The lack of more developments in DON research is consequence of the substantial analytical challenges inherent to it. DON concentrations are substantially lower than DOC concentrations, multiple chemical analyses are required for a single DON determination and inorganic N removal is a nightmarish undertaking. To measure DON concentrations, it is first necessary to obtain an accurate TDN concentration. The total TDN pool consists of an inorganic

fraction, composed of ammonium (NH_4^+), NO_3^- and NO_2^- and an organic fraction (DON), the composition of which is largely unknown (Bronk, 2002).

Westerhoff and Mash (2002) mention that the exact structural composition of organic nitrogen is still debatable, primarily due to analytical limitations and its incorporation into a wide range of molecular weights. Nevertheless Berman and Bronk (2003) identify the better-characterized constituents of the DON pool as urea, DFAA (dissolved free amino acids), DCAA (dissolved combined amino acids) and proteins, nucleic acids, amino sugars, and humic substances.

As said, much of the DON pool still remains uncharacterized chemically. Operationally, components of the DON pool have been divided into high molecular weight (HMW, usually >1 kDa) and low molecular weight (LMW) compounds. HMW DON includes proteins (such as enzymes, modified bacterial wall proteins, DCAA, nucleic acids – DNA and RNA) and humic like substances that have a relatively low N content. There is the added complication that some LMW and HMW DON compounds may be loosely held or adsorbed to humic substances (Berman and Bronk, 2003).

DON is considered, by Leenheer *et. al.* (2007), being primarily composed of degraded amino sugars, peptides and porphyrins.

Numerous N-containing compounds have been detected in wastewater effluents, including urea, aminoacids, LMW amines and chelating agents. In spite of this, the sum of identified compounds commonly accounts for less than 10% of the total DON present in wastewater effluents (Pehlivanoglu-Mantas and Sedlak, 2008).

3.4. Adverse effects

In many freshwater, marine, coastal, and estuarine environments a significant proportion of the TN pool (excluding N_2) is often associated with the DON fraction. Indeed, in many natural waters, DON concentrations are much higher than those of the total dissolved inorganic nitrogen (DIN) fraction, consisting of NH_4^+, NO_3^- and NO_2^- (Berman and Bronk, 2003).

The contribution of DON to the TN content of highly treated (as in nitrified and denitrified processes) wastewater effluent is relatively high and significant for watershed protection plans because mostly the plans for total maximum daily load account TN as the nitrogen parameter and do not consider DON as having a different

potential from inorganic nitrogen to cause eutrophication (Pehlivanoglu-Mantas and Sedlak, 2004).

As seen in section 1.1 and 3.2, eutrophication is known to be caused by the availability of excess nutrients, as N, in the water bodies such as lakes and rivers, which leads to low DO conditions and impacts severely estuaries and coastal waterways worldwide (Howarth and Marino, 2006).

Wastewater-derived DON has an important contribution to anthropogenic nitrogen inputs in the water bodies; however there is a lack of information about the bioavailability of wastewater-derived DON. The existing literature data about wastewater-derived organic nitrogen consists mostly of research conducted in the 1970s (Pehlivanoglu-Mantas and Sedlak, 2006).

It is stated and accepted that DIN is bioavailable to most aquatic microbes (counting also with bacteria and phytoplankton), though DON is related to a lower bioavailability, in particular to phytoplankton (Filippino *et. al.*, 2011). Nevertheless, recent research has shown that a variety of DON compounds are directly bioavailable to natural plankton communities (reviewed in Berman and Bronk 2003; Mulholland and Lomas 2008).

As reviewed in Pehlivanoglu-Mantas and Sedlak (2006), DON compositions most likely to influence the variability in its bioavailability in natural waters. Where heterotrophic bacteria and/or marine and freshwater algae have a direct uptake of free amino acids urea and nucleic acids. Other forms of DON, such as humic substances, are less easily used to support growth of algae in N-limited systems.

Different components of DON are considered indeed bioavailable to microorganisms (including phytoplankton, cyanobacteria, and bacteria) living in estuaries, either directly or after physical, chemical, and biologically-mediated reactions in the receiving waters and during transport along an estuarine gradient (Mulholland *et. al.*, 2007). Mulholland *et. al.* (2007) also points out the possibility of organic material change with photochemical reactions and readily convert recalcitrant (resistant to biological transformations) compounds into reactive material. Biologically non-reactive DON through photochemical reactions can release biologically available nitrogen and also convert DON to inorganic nutrients such as nitrite and ammonium.

The bioavailability of DON has high significance when is to characterize the significance of DON discharge with wastewater effluents. As Pehlivanoglu-Mantas and

Sedlak (2004, 2006) demonstrated, although most DON is recalcitrant, approximately 10% of the wastewater-derived DON was available to algae in the absence of bacteria, whereas the bioavailable fraction increased to 60% in the presence of bacteria. Bacteria in the cycling of wastewater-derived organic nitrogen potentiate DON bioavailability and with bacteria, approximately 25% of the DON is labile and becomes available for algae growth. Also, with Pehlivanoglu-Mantas and Sedlak research, there was the identification of potent carcinogenic nitrogenous disinfection by-products, such as nitrosamines, nitromethanes and NDMA and their resistance to biodegradation in receiving streams, and therefore of concern for downstream drinking water treatment.

Pehlivanoglu-Mantas *et. al.* (2004) makes clear that although disinfection of water offers protection against waterborne infectious diseases, it also increases the risk of other diseases such as cancer due to the formation of disinfection by-products. The presence of DON in wastewater effluent is of a high relevance for indirect potable wastewater reuse. Whether this reuse is intentional or not wastewater-derived ON may serve as disinfection by-products precursor during wastewater disinfection or possible drinking water treatment with chlorine disinfection (Pehlivanoglu-Mantas, 2004; Lee *et. al.*, 2007; Krasner *et. al.*, 2009). Besides disinfection by-products, Pehlivanoglu-Mantas points out that various different organic compounds, including humic substances, amino acids, and proteins, have been shown to form trihalomethanes (THMs) and dihaloacetic acids (DHAAs) upon chlorination and therefore it is likely that chlorination of organic compounds in wastewater effluent could result in the production of similar by-products.

Moreover, the process efficiency of chlorination and decholorination can be affected by wastewater-derived DON (Pehlivanoglu-Mantas *et. al.*, 2004).

3.5. Measurement techniques

The study of DON has its challenge and difficulty in the determination of concentrations. The lack of sensitive and precise techniques to quantify total DON concentrations and several of the known DON constitutes makes it a slow and complex process.

To measure total DON concentrations, presently, the majority of the methods depend on the TDN concentration and on the subtraction of the DIN concentrations, previously measured (the sum of NH_4^+, NO_3^-, NO_2^-). Consequently this approach combines the

analytical errors of three analyses TDN, NH_4^+, NO_3^- and NO_2^- (Berman and Bronk, 2003). TN is composed of organic nitrogen, ammonia nitrogen, nitrite and nitrate. The relationships are shown below:

Total Nitrogen = Organic Nitrogen + Ammonia Nitrogen + Nitrate + Nitrite

Total Kjeldahl Nitrogen = Organic Nitrogen + Ammonia Nitrogen

Total Nitrogen = TKN + Nitrate + Nitrite

Thus, the DON determination's accuracy relies strongly on how accurate are the methods used to determine each inorganic species and also is dependent on the fraction of DIN in TDN. For DON determination there are three sequential steps, where first the measurement of the inorganic nitrogen concentrations is made, after the TDN concentration and finally there is the subtraction of both concentration values (Lee and Westerhoff, 2005).

To measure TDN, not being possible to measure it directly, it is necessary a preparatory digestion step, either chemical or by combustion (Vandenbruwane *et. al.*, 2007). Also, it is necessary a previous sample treatment, where a filtration usually through 0.45µ membrane filters is made (Bratby *et. al.*, 2008). There is the conversion of DON and DIN to a single inorganic species (for example NO_3^-) and finally DON is the concentration difference (Westerhoff and Mash, 2002):

$$DON = TDN - DIN$$

$$DON = TDN - [NO_2^-] - [NO_3^-] - [NH_4^+]$$

There are three general types of digestion methods; the wet chemical oxidation (as persulfate oxidation), photolytic oxidation and high-temperature combustion (Westerhoff and Mash, 2002; Lee and Westerhoff, 2005). See **Table 3.1** for more details.

Total Kjeldahl Nitrogen (TKN) is the oldest method used (Vandenbruwane *et. al.*, 2007), where there is the conversion of all DON in ammonia and its subtraction from the TKN. However it has, generally, a low precision (method detection limit of 0.1-0.2 mgN/L) and the flaw of an incomplete oxidation of some nitrogenous compounds (Lee and Westerhoff, 2005).

The existent methods for digestion of organic nitrogen to inorganic nitrogen are present in **Table 3.1**, adapted from Westerhoff and Mash (2002).

Table 3.1 – Methods for total dissolved nitrogen analysis. (Adapted from Westerhoff and Mash, 2002).

Method	Description	Species measured	Observations
Kjeldahl nitrogen	Digestion (at 182–210°C) of sample in H_2SO_4 in the presence of a catalyst (usually Hg) and a salt (typically K_2SO_4)	NH_4^+, HSO_4^-	Azide, azine, azo, hydrazone, nitro, nitroso, oxime, and semicarbozone are unreactive functional groups. DON is converted and measured by titration, colorimetry or ion-selective electrode (APHA, 2005); high ammonia concentrations decrease the sensitivity and precision (Pehlivanoglu and Sedlak, 2006)
Dumas method	Combustion in CO_2,reduction of NO_x followed by CO_2 removal	Volumetric quantity of N_2 produced	Dry, solid samples required
Alkaline persulfate oxidation	Autoclave digestion under alkaline conditions in the presence of $S_2O_8^{2-}$	NO_3^-	Interference by organic carbon concentrations greater than 150 mg/l and with analytical ranges from 0.03 to 5.00 mg-N/L (Patton and Kryskalla, 2003)
	Microwave digestion under alkaline conditions in the presence of $S_2O_8^{2-}$		Proteins problematic; antipyrine non-quantifiable
	Boiling digestion under alkaline conditions in the presence of $S_2O_8^{2-}$		EDTA and antipyrine not efficiently oxidized
	UV digestion under alkaline conditions in the presence of $S_2O_8^{2-}$		Hetercyclic compounds show low recovery
Pyrolysis oxidation	High temperature oxidation at1100□C	NO, NO_2^- and N_2	Pyrazole and azoxy compounds recovery poor; poor recovery of azo, nitro and nitroso compounds
High temperature catalytic oxidation	High temperature oxidation at 680□C in the presence of acatalysis	NO, NO_2^- and N_2	Does not oxidize always certain recalcitrant forms of organic nitrogen. Low values when compared to the persulfate digestion method, possible consequence of recalcitrant compounds as urea (Pehlivanoglu and Sedlak, 2006)
Photooxidative degradation	UV-oxidation, typically assisted by the presence of an oxidizing agent ($S_2O_8^{2-}$ or H_2O_2)	NO_3^-	Bigger disadvantage is inconsistency (Bronk *et. al.*, 2000). Bronk *et. al.* (2000) verifies it with the UV method performed on different UV machines and within the same machine with different UV lamps.
	UV-oxidation in the presence of TiO_2 or TiO_2/Pt catalyst	NO_2^-, NO_3^- and NH_4^+	High salt content interference

3.6. Removal techniques

While advanced treatments of wastewaters have not historically considered DON removal as a major goal, as previously seen, it has been gaining an increased importance (Chen *et. al.*, 2010).

Now, WWTP that are planning to achieve very low nutrients levels need do consider methods for the removal of effluent fractions hitherto not taken into account (Arnaldos and Pagilla, 2010).

Due to the small percentage of compounds that are known to compose DON (less than 10%) and the rest being vary heterogeneous, there is the need to investigate tertiary technologies that will target DON specifically (Urgun-Demirtas *et. al.*, 2008).

Previous studies have evaluated the removal of DON in the effluent and the effectiveness of these processes. In the study of Randtke and Mccarty (1977) physical-chemical processes were tested for DON removal in the Palo Alto, California effluent. The Palo Alto facility effluent had a DON concentration, in bench scale tests, of 1.3 mg/l (WERF, 2008). It was used chemical treatment for DON removal and typically, chemical precipitation is obtained through the use of lime or a metal salt such as aluminum sulfate or ferric chloride (US EPA, 2009). There was a 33% of removal with lime, 28% with 200-300 mg/l alum, and 40% with 200-300 mg/l ferric chloride. For cation and anion exchange less than 13%. About 71% of the effluent DON was removed with activated carbon adsorption (WERF, 2008).

Nevertheless, Pehlivanoglu-Mantas and Sedlak (2008) showed through solid-phase extraction that the unidentified DON was relatively hydrophilic and due to this characteristic, DON is notlikely to be removed by adsorption onto activated carbon. Activated carbon is most effective at removing less polar material. Molecules of higher polarity tend to be less absorbable, bind water more tightly, and are more soluble (Bratby *et. al.*, 2008). On the contrary, aluminum coagulation has been proved to remove nitrogenous organic compounds present in molasses wastewaters (Dwyer *et. al.*, 2009) and surface waters (Lee and Westerhoff, 2006).

For aluminum coagulation and lime softening Chen *et. al.* (2011) obtained until 25% of DON and DOC removal. Especially due to the low specific ultraviolet absorption values (<2 L/mg-m), there is a poor DON adsorption onto aluminum floc (coagulation) or calcium carbonate solids (lime softening). Likewise, there is a low adsorption capacity of DON onto activated carbon.

In Arnaldos and Pagilla (2010) study, for aluminum coagulation using a dose correspondent to 3.2 mg Al (III)/L, was achieved a maximum DON removal of 69%. This percent removal represents the fraction of effluent DON amenable to removal by enhanced coagulation and microfiltration.

Also, in Lee and Westerhoff (2006) study the removal of DON in drinking water, with aluminum sulfate coagulation was of 5 to 40% depending on the dosage of aluminum sulfate and cationic polymer.

Even though the majority of the DON has LMW, microfiltration or nanofiltration may not be very useful in removing wastewater-derived DON due to the fouling problems often encountered in these filtrations (Pehlivanoglu-Mantas and Sedlak 2008). Also, as reviewed in Bratby *et. al.* (2008), Muller (2006) with an industrial case of study, reported that for a WWTP with a flow of $3\times10^6$of gallons per day (0.13 m^3/s), the capital cost of a membrane plant (microfiltration or ultrafiltration followed by reverse osmosis) for effluent DON removal would be up to about $\$26\times10^6$. Annual operating costs would be approximately $\$2.7\times10^6$. If a granular activated carbon treatment system were to be implemented, operating costs would increase to $\$11\times10^6$per year.

There are other possible methods for DON removal, as Chen *et. al.* (2011) refer DON seems to be partially removed with in-situ biological treatment using soil systems or rivers. There is the demonstration of Amy and Drewes (2007) with soil-aquifer treatment achieve DON removals from 50 to 75%, with effluents from Mesa wastewater reclamation plant (WRP) and Tucson WRP (Bratby *et. al.*, 2008).

The removal of natural organic matter (NOM) was extensively studied through chemical coagulation or precipitation. Aquatic NOM has its major composition of 'humic substances' (Arnaldos and Pagilla, 2010). As 90% of the wastewater effluent DON may also be composed of humic substances (Pehlivanoglu-Mantas and Sedlak, 2008), the removal of DON can be investigated to be the same coagulant addition as those used in NOM removal (Arnaldos and Pagilla, 2010).

Nevertheless, the efficiency of enhanced coagulation depends largely on the water or wastewater characteristics and constituents. The fractions of DON and NOM with a HMW are generally easier to remove, thus the LMW fractions are generally more difficult to remove, tending to be more recalcitrant. The effluent wastewater-derived DON has a LMW in general, being so difficult to remove to a great extent by coagulation. It is the predominantly LMW of DON in wastewater effluents which makes

its removal difficult (Brabty *et. al.*, 2008). Likewise, in Bratby *et. al.* (2008) study there is the reference of DON removal to about 31%, through enhanced coagulation, the initial DON concentration.

4. Materials and Methods

This chapter describes the set-up used for the batch experiments, the analytical methods and the used materials.

4.1. Experimental SET-UP

The experimental part consisted of two types of denitrification batch tests: with suspended biomass and attached biomass. Both types of batch tests were carried out in a 20 L reactor, where anoxic conditions were provided with introduction of N_2 gas in the system, when necessary. A dissolved oxygen (DO) probe was connected in recirculation with the reactor to control the absence of oxygen. The activated sludge (in the case of suspended biomass tests) or the biological carriers and treated effluent (in the case of attached biomass tests) were mixed at a constant speed, through a static mixer.

The Harnaschpolder (HNP) WWTP is one of the largest WWTP in Europe, designed for 1.3×10^6 inhabitant equivalents. It is located in the border of Rijswijk and Delft and treats in average 255 000 m^3 of waste water per day. The treatment process starts with a bulky waste removal, followed by a pre-sedimentation tank, an active sludge tank (biological treatment), a post-sedimentation tank and sludge treatment. For the denitrification tests the activated sludge and treated effluent were collected from HNP WWTP.

4.2. Batch tests with activated sludge

For the batch tests with suspended biomass, activated sludge from the HNP WWTP was used.

Batch test preparation

1. The collection of the activated sludge was made at the HNP WWTP in the previous day of the test and transported to the laboratory.
2. The sludge was aerated for at least one hour, in order to deplete all biodegradable COD, **Figure 4.1**.

Figure 4.1 – Activated sludge aeration

Batch test

1. The activated sludge was introduced in the reactor and N_2 gas is provided in case that DO was not 0 mg/l, **Figure 4.2**.

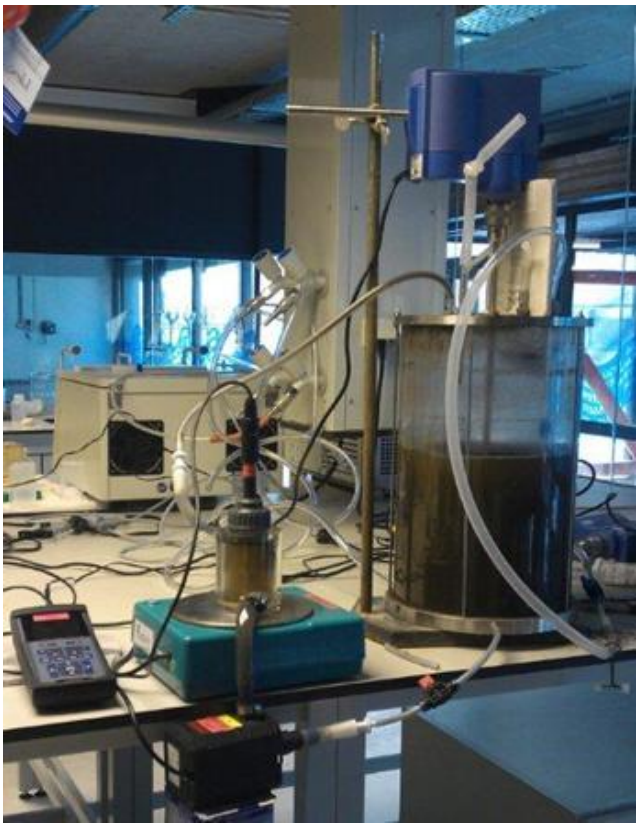

Figure 4.2 – Denitrification batch test with activated sludge

2. A constant speed of 43 rpm was set, through a static mixer.
3. When DO concentration of 0 mg/l was achieved, NO_3 concentration was measured.
4. In the case that NO_3 would not be 0 mg/l, Sodium Acetate Trihydrate ($C_2H_9NaO_5$) solution (SAT) was added to the reactor in order to denitrify all NO_3 existent in the activated sludge. The amount of added SAT was calculated in order to provide a COD concentration according to the following equation:
   $COD = 2.86xNO_3 + 1.71xNO_2 + DO$
5. When a NO_3 concentration of 0 mg/l was achieved, samples were taken for DON determination.
6. SAT was added to the reactor in order to provide COD enough to denitrify the initial NO_3 concentration of 10 mg/l.
7. After 10 minutes of SAT addition (to guarantee homogenization) a sample was collected for new measurement of COD and DON determination.
8. Sodium nitrate ($NaNO_3$) solution was added to the reactor to achieve an initial concentration of 10 mg/l NO_3.
9. After 10 minutes (to guarantee homogenization of SAT), a sampling collection with a frequency of 4 min started.
10. Each sample consisted of:
    10.1. One 50 ml flask, which was left in the fridge at 2ºC, for later measurement of biomass.
    10.2. Three 100 ml flasks which were kept in dry ice for 3.5 minutes, for a fast cool down, and afterwards into a fridge at 2ºC.
11. The pH and temperature were measured along the test through a portable meter.
12. Extra samples were collected during the test once in a while and immediately measured for NO_3 to make sure when the denitrification was finished.

Samples treatment

1. Samples that were conserved in the fridge were centrifuged (Sorvall ST16R, Sysmex, The Netherlands) by order of collection for seven minutes at 1200 rpm.
2. After centrifugation, the samples were decanted and frozen for later measurement of NO_3^-; NH_4^+ and NO_2.
3. Before measurement the samples were melted at room temperature and filtered by membrane filtration through 1.2 µm pore size followed by 0.45µm.
4. Part of the filtrate was immediately measured for NO_3^-; NH_4^+ and NO_2, and part was acidified to pH 2 with acid chloridric.

5. Part of the acidified sample was conserved in the fridge for DOC measurement and part was frozen for later DON determination.

4.3. Batch tests with *kaldnes* carriers

The batch tests with attached biomass were performed with secondary effluent from the HNP WWTP and with *kaldnes* biological carriers, for attached growth.

Before initiating the batch tests with attached biomass, the *kaldnes* carriers were fed with synthetic wastewater for 90 days, in order to create a biofilm that could perform denitrification.

Feeding of biological carriers for biofilm growth

Synthetic wastewater was prepared by adding organic carbon, nitrate, phosphorus, micronutrients and a buffer solution (for pH regulation) to tap water. Table 4.1 and 4.2 present the composition of synthetic wastewater and buffer solution, respectively.

Table 4.1 – Composition of the Synthetic Wastewater

Synthetic Wastewater (10L)	
$C_2H_9NaO_5$	34.16g
$NaNO_3$	2.91g
K_2HPO_4	0.173g
Trace solution	150.6ml
Intermediary solution	0.1ml

Table 4.2–Composition of Buffer Solution

Ingredient	Concentration [g/l]
Na_2HPO_4	3.46
$NaH_2PO_4{\cdot}2H_2O$	1.93

The *kaldnes* were fed everyday with synthetic wastewater for a period of 90 days before the first *kaldnes* test. For further information about the preparation of the synthetic wastewater see appendix III.

Batch test preparation

To evaluate the influence of mixing velocity in DON concentration, the batch tests with attached biomass were carried out at two different mixing velocities: velocity 1 with 43 rpm, and velocity 2 with 108 rpm. Velocity 2 was selected to be 2.5 times higher than velocity 1. A higher velocity difference was not possible due to static mixer restrictions.

1. The collection of the secondary effluent was made at the HNP WWTP in the same day of the test and transported to the laboratory.
2. The effluent was mixed to ensure its homogeneity and measured for NO_3^-, NO_2 and NH_4^+.
3. Three samples were collected for DON determination.
4. The first sample was immediately frozen, the second was filtrated by 1.2 µm pore size and frozen and the third sample was filtrated through 1.2 µm, followed by 0.45 µm pore size and frozen.
5. The *kaldnes* carriers were separated from the feeding solution through the use of a metallic sieve, **Figure 4.3**.

Figure 4.3 – Separation of kaldnes carriers from the feeding solution

Kaldnes washing

1. The *kaldnes* was transferred to a bucket where 10 L of demineralized water were afterwards discharged for washing of the *kaldnes*.
2. The *kaldnes* and demineralized water were mixed at velocity 1 through a static mixer and a bucket bottom discharge was opened in order to continuously flush the washing water out of the system. The aim of washing the *kaldnes* was to assure the elimination of feeding solution remaining.
3. The washing is repeated for 9 times.
4. At the 9th washing a sample of 100 ml was collected, filtered by 0.45 μm pore size for measurement of TN in triplicate. This step was made to make sure that all accumulated TN of the feeding solution was eliminated.

Solids measurment

1. 200 *kaldnes* units were collected before starting each test with low rpm for measurement of total suspended solids (TSS) and total volatile solids (VSS). The *kaldnes* units were stored in flasks containing demineralized water and kept in the fridge until further sonication.

Batch test

1. The batch test reactor was filled with 8 L of *kaldnes* and a metallic net was used inside the reactor in order to keep all *kaldnes* submerged during the test. Otherwise, the water level decreases in the reactor, due to sampling, would lead to accumulation of *kaldnes* on the top of the reactor.
2. A constant speed of 43 rpm or 108 rpm was set, through a static mixer.
3. The secondary effluent was introduced in the reactor and N_2 gas was provided in case that DO was not 0 mg/l, **Figure 4.4**.
4. When DO concentration of 0 mg/l was achieved, NO_3 concentration was measured.
5. In the case that NO_3 is not 0 mg/l, SAT is added to the reactor in order to denitrify all NO_3 existent in the activated sludge. The amount of added SAT was calculated in order to provide a COD concentration according to the following equation:
   $COD = 2.86 \times NO_3 + 1.71 \times NO_2 + DO$
6. When a NO_3 concentration of 0 mg/l was achieved, samples were taken to measured COD and DON determination.
7. SAT is added to the reactor in order to provide COD enough to denitrify the initial NO_3 concentration of 10 mg/l.

8. After 10 minutes of SAT addition (to guarantee homogenization), a sample was collected for new measurement of COD and DON determination.
9. $NaNO_3$ solution was added to the reactor to achieve an initial concentration of 10 mg/l NO_3.
10. A sampling collection with a frequency of 4 minutes starts immediately.

Figure 4.4 – Denitrification batch test with kaldnes carriers

11. Each sample consisted of:
    11.1. One 180 ml flask, which was kept in dry ice for 3.5 minutes, for a fast cool down, and afterwards into a fridge at 2ºC.
12. The pH and temperature were measured at the beginning and end of the test through a portable meter.
13. Extra samples were collected during the test once in a while and immediately measured for NO_3^- to make sure when the denitrification test was finished.

Samples treatment

1. Samples that were conserved in the fridge were filtrated by order of collection by 1.2 µm and 0.45 µm pore size and frozen afterwards.

2. Samples were melted at room temperature; part of the sample was immediately measured for NO_3^-, NO_2 and NH_4^+ and part was acidified to pH 2 with hydrochloric acid.
3. Part of the acidified sample was conserved in the fridge for DOC measurement and part was frozen for later DON determination.

4.4. Preparation of solutions

$NaNO_3$ [30.35g/l]

Sodium nitrate is dried in the oven at 105ºC for 24h.

15.175g of dried $NaNO_3$ are dissolved in 500ml of demineralized water.

The dissolved solution is transferred to a volumetric flask and demineralized water is added in order to obtain a final solution of 500ml.

SAT [100g/l]

25g of sodium acetate trihydrate are dissolved in a beaker with demineralized water.

The dissolved solution is transferred to a volumetric flask and demineralized water is added in order to obtain a final solution of 250ml.

4.5. Analytical measurements

For the determination of the denitrification curves, NO_3^-, NO_2 and NH_4^+ were measured through MERCK cuvette tests (Darmstadt, Germany). The MERCK tests are based on colorimetric tests according to standard methods (APHA, 2005).

For purposes of DON determination, NO_2 and NH_4^+ were measured by Hach Lange cuvette tests (Dusseldorf, Germany) and NO_3^- absence was checked through ion chromatographic method with the use of DIONEX ICS - 1000 Ion Chromatography System, with an AS-DV auto sampler unit.

DON concentration was determined by subtracting the inorganic nitrogen (NO_3^-, NO_2 and NH_4^+) to TDN (TN measured after filtration through 0.45 μm pore size). TN was measured through persulfate digestion according to standard methods (APHA, 2005)

COD concentration was measured using Merck kits (Darmstadt, Germany) and DOC was measured using water extraction total organic carbon measurement method with the use of TOC/TN analyzer (SHIMADZU Corporation, Japan).

The TSS and VSS concentration for the activated sludge was performed as described in the standard methods (APHA, 2005).

The assessment of the TSS and VSS concentration on the *kaldnes* biological carriers was performed as follows: the attached biomass was removed from the 200 biological carriers by putting them in a flask with demineralized water that was placed in an ultrasonic bath (Branson 5510) for six hours. After the biological carriers were rinsed with demineralized water, a sample of the water containing the biomass of the *kaldnes* was collected. This sample was used to measure TSS and VSS according to standard methods (APHA,2005).

TSS and VSS were calculated according to standard methods and considering the specific surface area of the type of *kaldnes* carriers used, and the number of *kaldnes* units per liter (see **appendix II**) in the reactor.

5. Results and discussion

This chapter contains the results of the batch experiments performed for suspended and attached biomass. These results are discussed and compared with literature.

This chapter is divided in two types of batch tests: with activated sludge (**section 5.1**) and *kaldnes* carriers (**section 5.2**).

The **section 5.2** is divided in results obtained with low and high mixing velocity.

5.1. Batch tests with activated sludge

The concentrations of NO_3^-, NO_2 and NH_4^+during the three tests of activated sludge are shown in **Figure 5.1**.

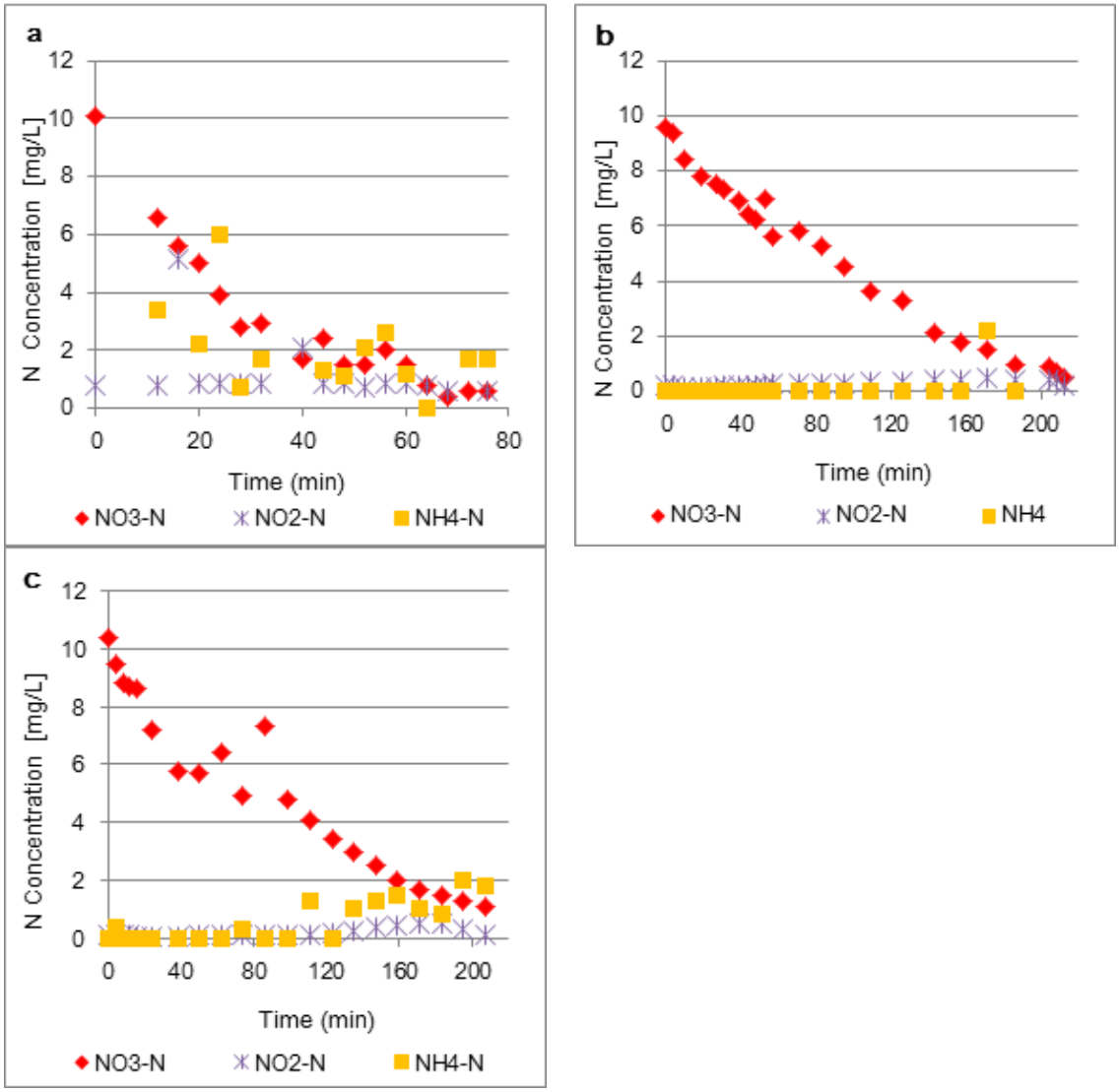

Figure 5.1 – Inorganic nitrogen concentrations over time during activated sludge tests a, b and c.

The concentrations of NO_2 remained most of the time close to 0 mg/l which agrees with the general consideration that the reduction rate of nitrite is higher than nitrate reduction rate and high enough so nitrite accumulation will not occur during the denitrification process (Wilderer *et. al.*, 1987). Concentrations of NH_4^+ were mainly close to zero as well, which was expected since the activated sludge was aerated before the tests, resulting in nitrification of the remaining NH_4^+.

Table 5.1 shows the calculated NO_x–N specific denitrification rates (SDNR), obtained for the three activated sludge tests.

Table 5.1 – SDNR for activated sludge tests

Batch test	Denitrification rate		VSS [g/l]	T (^{0}C)	pH
	[gNO_3^--N/KgVSS.d]	[gNO_x-N/KgVSS.d]			
a	70	71	2.58	20 ± 0.6	7.0 - 8.0
b	22	22	2.79	21 ± 0.5	6.0 - 7.0
c	28	28	2.28	19 ± 0.5	7.0 - 8.0

The denitrification efficiency from each batch test was assessed by the specific denitrification rate. For the first activated sludge test (test **a**), the calculated SDNR was 70 gNO_3^--N/(KgMLVSS.d), which is in the range of 40 to 420 gNO_3^--N/(KgMLVSS.d) observed for pre-anoxic tanks in full scale installations (Metcalf and Eddy, 2004) or close to the range of 72 to720 gNO_3^--N/(KgMLVSS.d), observed for anoxic batch tests (Ekama *et. al.*, 1986).

For the last two tests of activated sludge (**b** and **c**), the SDNR are 22 and 28 gNO_3^--N/(KgMLVSS.d). Assuming that VSS/TSS is usually 70%, such SDNR correspondent to 11 to 42 g N/(KgMLVSS.d), observed by Gerber*et. al.* (1986) in denitrification batch tests under anoxic conditions, which cover the values obtained for tests **b** and **c** of activated sludge.

Acetate or methanol addition are indicated in the literature by Henze *et. al.* (1994) and Dold *et. al.* (2008) as producing the maximum SDNRs in BNR processes. Regarding the use of acetate, Cherchi *et. al.* (2009) observed variable SDNR which were attributed to different sludge sources and environmental factors that affect biological processes (as pH and temperature). The temperature and pH observed for the three tests with activated sludge were in the range of 19 to 21ºC and 6 to 8, respectively. The big difference between the first test and the last two might be related to the specificities of the biomass present in the collected activated sludge.

The graphs of NO_x concentrations during denitrification for the activated sludge tests **a**, **b** and **c**, are presented on **appendix I (Figure I.I).**

Denitrification is mainly accomplished by heterotrophic bacteria and is strongly dependent on the availability of organic carbon, which serves as an energy source and electron donor of the denitrification process (Lin *et. al.*, 2009). Organic carbon can be expressed as the chemical oxygen demand (COD) to N ratio, also named as COD/N (Peng *et. al.*, 2007), which is the carbon-use-to-nitrate-consumption ratio (Cherchi *et. al.*, 2009). As common procedure, denitrification tests are evaluated in terms of C/N ratio, which indicates the amount of carbon used (acetate in the current case) for the removal of nitrate (Dold *et. al.*, 2008).

COD and DOC concentration are expected to decrease along with the decrease of NO_3 or NO_x. However, the resultant concentrations did not show a clear decrease for any of the three tests (graphs are shown in **appendix I.II** and **I.III**). Instead, small variations occurred within a constant interval which could be the result of a measurement error. For this reason, the COD oxidized through these batch tests could not be accounted to quantify the ratio of COD:NO_3^- of the activated sludge tests.

Figure 5.2 presents the results for the concentrations of DON and NO_x-N in the three tests. The vertical bars in the graphs present the standard deviation obtained from three DON results, calculated from the subtraction of a single measurement of inorganic N to the average of three measurements of TDN determinations.

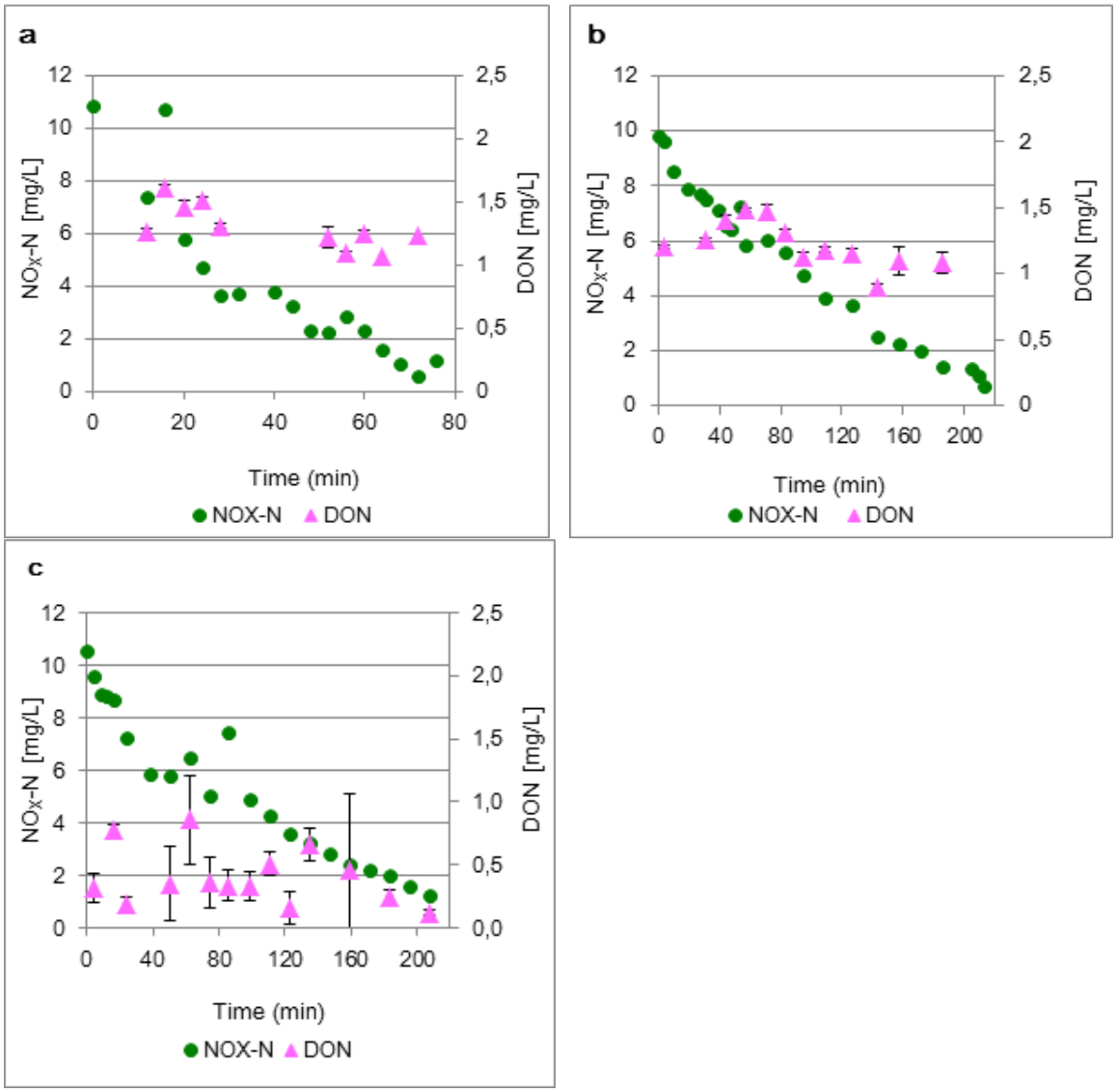

Figure 5.2 – Comparison between the NO_x concentrations and DON concentrations in the activated sludge tests a, b and c.

As observed in **Figure 5.2**, the concentrations of DON vary within 0.1 to 1.6 mg/l.

Looking at the tests **a**, **b** and **c** it is observed that DON tends to be constant with denitrification.

DON concentrations observed during the batch tests very between 0.1 to 1.6 mg/l. The measured DON values are comparable with the values obtained by Makinia *et. al.* (2011). The author measured organic nitrogen in an activated sludge batch test before and after four hours of anoxic conditions (initial and final values) and Makina *et. al.* (2011) observed a decrease of DON (called as CON by the author) from 5 to 2 mg/l. However, in this study several measurments are done during the denitrification of 10 mg N/l.

Several other authors have made previous DON determinations. DON concentration ranges of 0.7 to 1.8 mg/l (Liu *et. al.* 2012) and 0.7 to 2.1 mg/l (Pehlivanoglu-Mantas and Sedlak, 2008) in treated wastewater; of 1.0 to 2.5 mg/l for trickling filter effluent (Evans *et. al.*, 2004; Murthy *et. al.*, 2006; Pagilla *et. al.*, 2006) and 1 to 2 mg/l for activated sludge plants (Pagilla *et. al.* 2011).

Comparing the DON levels between tests, the last test shows much smaller concentrations with a maximum of 0.86 mg/l. This difference could be attributed to a different type of sludge, which contained originally a very low concentration of DON.

5.2. Batch tests with *kaldnes* carriers

The results of the batch tests with attached biomass are shown for the two studied velocities: velocity 1 and 2.

The objective of different mixing velocities was to induce different shear stress conditions in the biomass. The increase of shear stress may lead to an increase of biological stress which can result in the production of EPS, and consequently, to the increase of DON, as explained in chapter 1.

Low velocity

The following **figure 5.3** shows the curves obtained for NO_3^-, NO_2 and NH_4^+ for the velocity 1 (43 rpm).

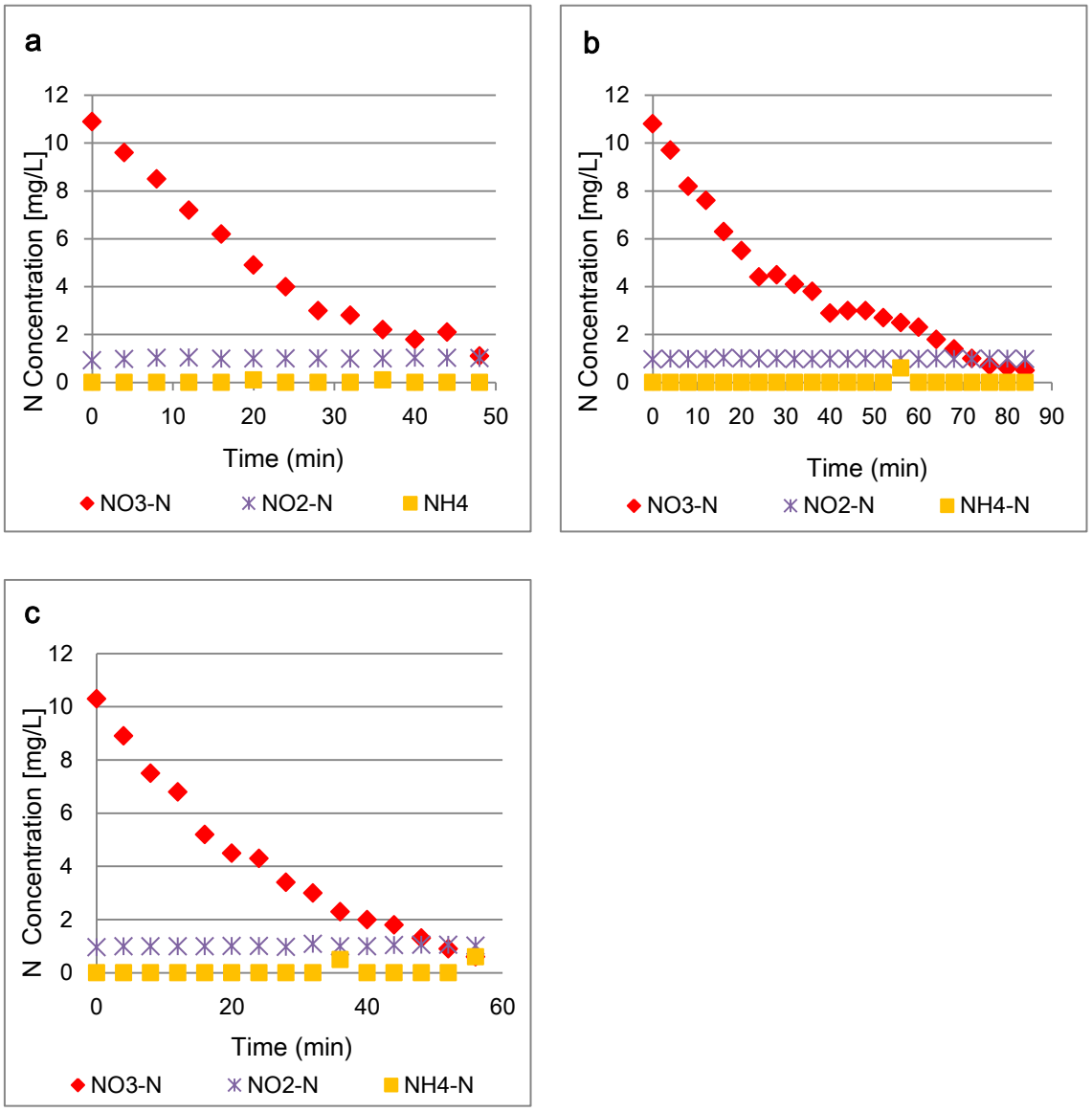

Figure 5.3 – Inorganic nitrogen concentrations over time during the low velocity *kaldnes* tests a, b and c.

As observed for the activated sludge tests, the concentrations of NO_2 and NH_4^+ remain mostly close to 0 mg/l. Regarding the activated sludge tests, the denitrification process with *kaldnes* is faster.

All tests have a similar run, translated by the SDNR, as showed in the **Table 5.2**

Table 5.2 – SDNR for kaldnes tests with velocity 1

Batch test		Denitrification rate		VSS [g/l of effluent]	T (^{0}C)	pH
		[gNO_3^--N/KgVSS.d]	[gNO_x-N/KgVSS.d]			
Velocity 1	a	160	159	1.84	24 ± 0.5	8.00 – 8.43
	b	132	131	1.34	25 ± 0.5	8.10 – 8.31
	c	166	165	1.50	26 ± 0.5	8.12 – 8.27

The obtained SDNR values (159, 131 and 165 g NOx/(KgVSS.d)) are consistent with Zafarzadeh *et. al.* (2010). The author has quantified a maximum and an average of SDNR of 40.1 g NOx/(KgVSS.d) and 157g NOx/(KgVSS.d), respectively. The kinetics values obtained for the SDNR of the denitrification tests with attached biomass are found to be similar with the range of values reported in the literature.

The curves of NO_x concentration variations between the three tests are shown in **appendix I**. The denitrification rates are showed in the **Table 5.2** and based on the conditions which the tests were performed these values vary in a range of 131 to 165g NO_x-N/(KgVSS.d).

Figure 5.4 shows the variation of COD concentrations with the NO_x concentrations along the denitrification test.

The graph presents a COD decrease, as expected, while NO_x is removed with a ratio of 5 gCOD/gNO_x-N.

Only the first *kaldnes* test (test **a**) presents an expected decreasing COD curve and so this was the only test for which the COD/N ratio was calculated.

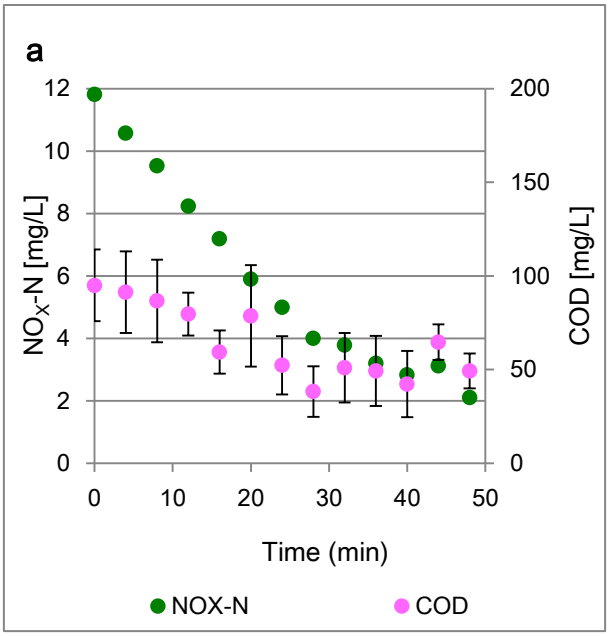

Figure 5.4 – Comparison between the NO_x concentrations and COD concentrations in the low velocity *kaldnes* test a.

For the other *kaldnes* tests (see **appendix I.II** and **I.III**) the results did not show the expected decreasing COD concentrations which can be resultant from measurement errors.

The obtained ratio of 5 $gCOD/gNO_x$-N for the *kaldnes* test **a** is within the range 4 to 15gCOD/gN indicated by Peng *et. al.* (2007), for partial or complete denitrification processes.

Figure 5.5 shows the obtained DON and NO_x concentrations in the three tests of velocity 1.

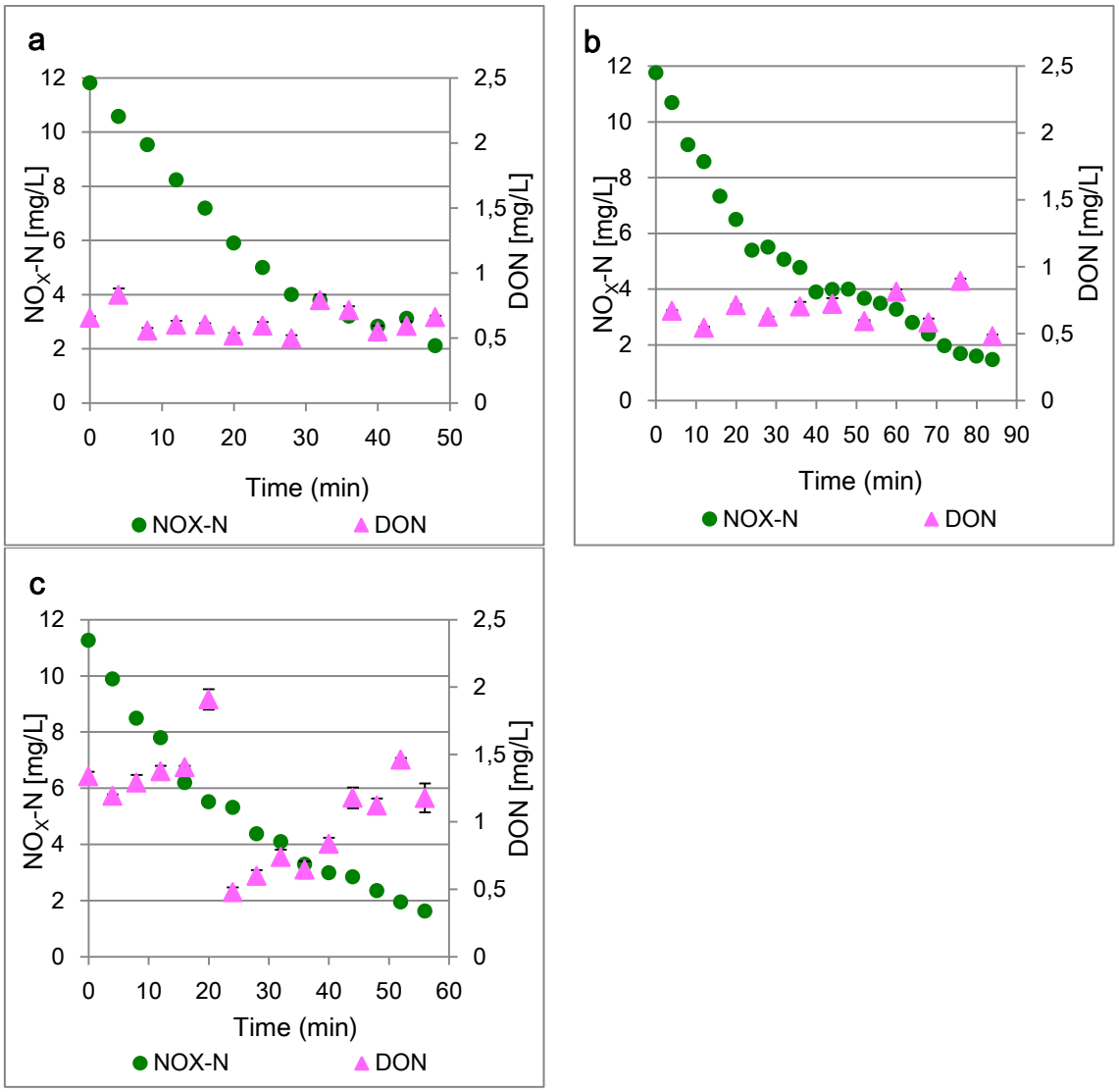

Figure 5.5 – Comparison between the NO_x concentrations and DON concentrations in the low velocity *kaldnes* tests a, b and c.

It is observed that there is not a decrease or increase of DON concentration but an oscillation in the range of values of 0.5 to 1.9 mg/l.

For these *kaldnes* batch tests (with velocity 1), the last test of the set has a higher variation in DON concentrations showing a range of values from 0.5 mg/l to 1.9 mg/l. The first two tests present a range between 0.5 and 0.9 mg/l. Even though the last test has a higher variation and range of values, the first two show a tendency for DON to remain constant.

High velocity

In this set of tests a velocity of 108 rpm was used.

Figure 5.6 presents the set of results of the nitrogen concentrations for the tests with velocity 2.

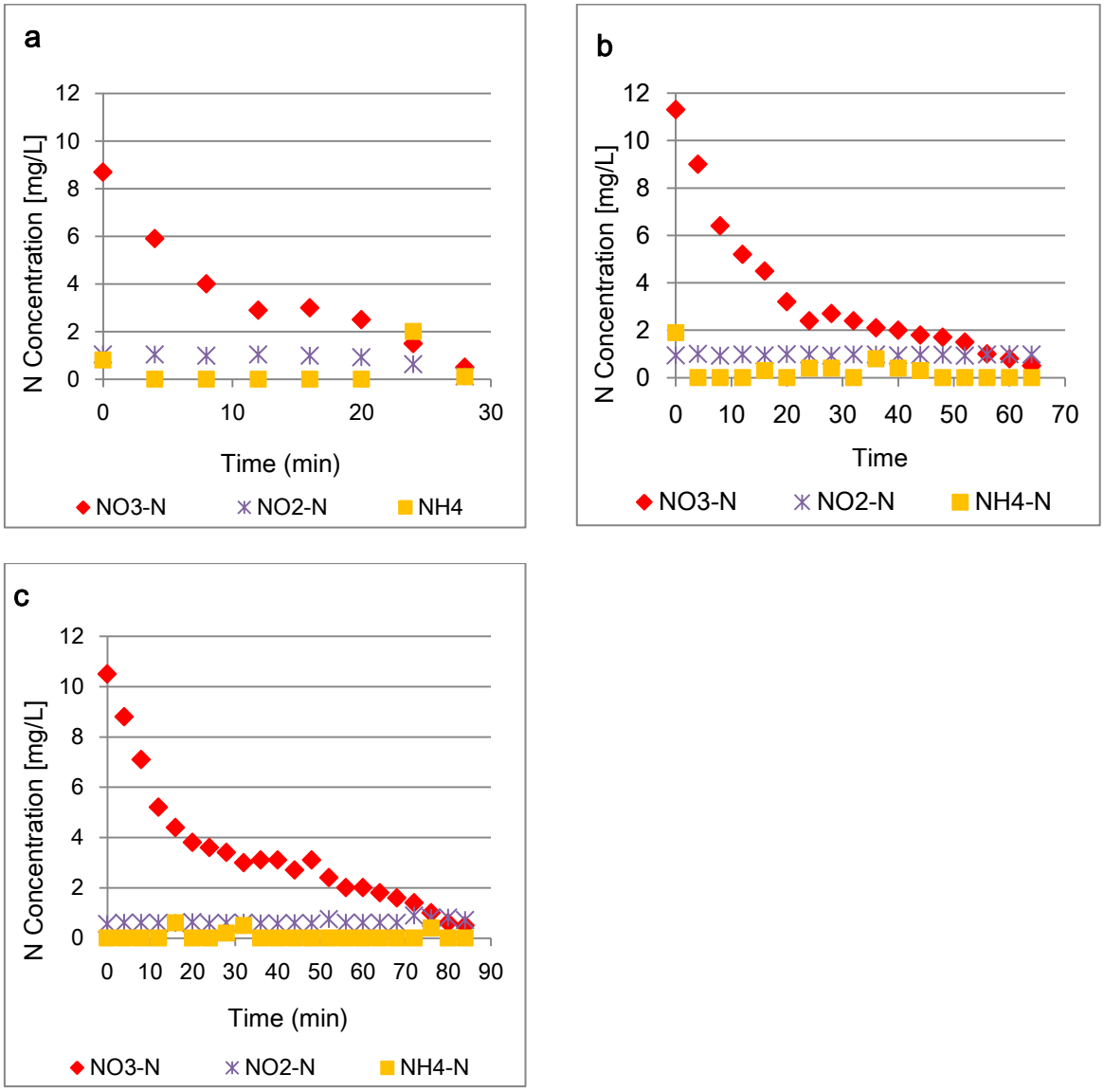

Figure 5.6 – Inorganic nitrogen concentrations over time during high velocity *kaldnes* tests a, b and c.

As expected, the concentration variation for NO_2 and NH_4^+ remains close to 0 mg/l along the denitrification.

Table 5.3 shows the denitrification rates obtained for the tests of velocity 2.

Table 5.3 – SDNR for *kaldnes* high velocity tests

Batch test		Denitrification rate		VSS [g/l of effluent]	T (ºC)	pH
		[gNO_3^--N/KgVSS.d]	[gNO_x-N/KgVSS.d]			
Velocity 2	a	230	255	1.84	26 ± 0.5	8.74 - 8.53
	b	181	180	1.34	26 ± 0.5	7.83 – 7.51
	c	114	112	1.50	26 ± 0.5	7.97 – 7.93

In appendix I (in Figure I.3) is presented the variation of NO_x concentrations during the denitrification process of the three tests.

Due to possible measurement errors, the COD concentrations do not decrease, as expected, with the decrease of NO_3^-. The results obtained for COD are presented in appendix I.II and I.III.

Figure 5.7 presents the concentrations of DON and NO_x in the three tests of velocity 2.

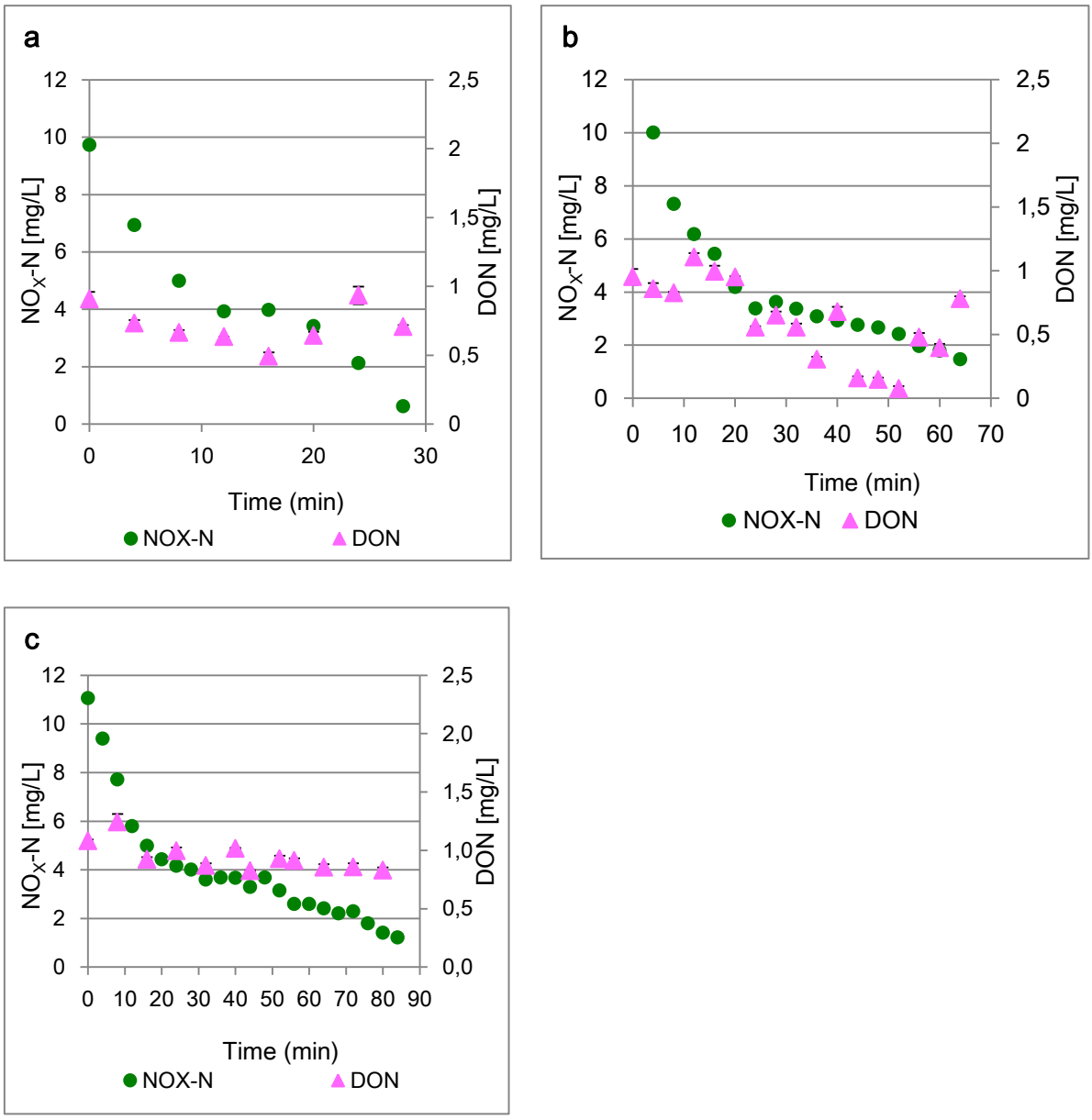

Figure 5.7 – Comparison between the NO_x concentrations and DON concentrations with high velocity *kaldnes* tests a, b and c.

The results are similar with the previous tests, where DON concentrations oscillate in a range of values from 0.1 to 1.1 mg/l.

Before the *kaldnes* batch tests that conducted to the presented results, previous tests were done but they had to be repeated due to interferences related to the persulfate digestion method, used for TN measurement.

In fact, the first tests showed very high TN results. It was discovered that such high results were obtained due to the interference of high COD in the samples. The high COD concentrations were not initially expected but it was found that they were caused by the accumulation of COD between tests. Such accumulation was due to the remaining of *kaldnes* feeding solution between tests. In order to overcome such interference a new *kaldnes* washing procedure was defined (as indicated in **chapter 4, section 4.3**).

From the previous analysis on the obtained denitrification rates, it is observed that the batch tests that were carried out in the present study were all comparable with literature studies. It is observed with the same initial concentration of nitrate (10mg NO_3-N/L) the denitrification occurs faster with *kaldnes* than with activated sludge. Thus, the tests are related with the literature indications where the volumetric removal rate is higher, meaning that the biomass of this process is more viable, active and specific than in a comparable activated sludge process (Ødegaard, 1999; Haandel and Lubbe, 2012).

The three *kaldnes* batch tests with velocity 2 are consistent among themselves having a range of values for DON concentrations from 0.1 to 1.1 mg/l. This set is consistent and similar between each test, again presenting the same range of values during the three experiments.

From the previous analysis on DON it is observed that the variation of mixing velocity does not affect DON variation during denitrification and for both, activated sludge and *kaldnes* tests, DON tends to be constant.

Although both batch tests give the understanding that DON concentrations along a denitrification test are stable, the range of values obtained is different, where for the *kaldnes* tests the range varies between 0.1 mg/l and 1.1 mg/l (with exception for the last test of *kaldnes* with velocity 1 where DON varies from 0.5 to 1.9 mg/l) and for the activated sludge, where the concentrations vary from 0.1 to 1.6 mg/l.

The pH values for all the tests are distributed in the range of 6.0 to 9.0. As reported in the literature, denitrification may occur within the pH range of 3.9 to 9.0, and the maximum nitrogen oxide reduction rate falls into pH 7.0 to 8.0 (Lin *et. al.*, 2009). The tests temperature varies from 19 to 27ºC and, as indicated, the best temperature conditions for the denitrifying bacteria are between 10 and 30ºC (Marx *et. al.*, 2010).

In this research a characterization of the secondary effluent used for the attached biomass denitrification tests was also made. As described in the **chapter 4, section 4.3**, organic nitrogen was measured for each test effluent without filtration, after filtration by 1.2 µm and after filtration by 0.45 µm pore size.

The following **Figure 5.8** presents the summary results of the DON determinations made for the effluent samples.

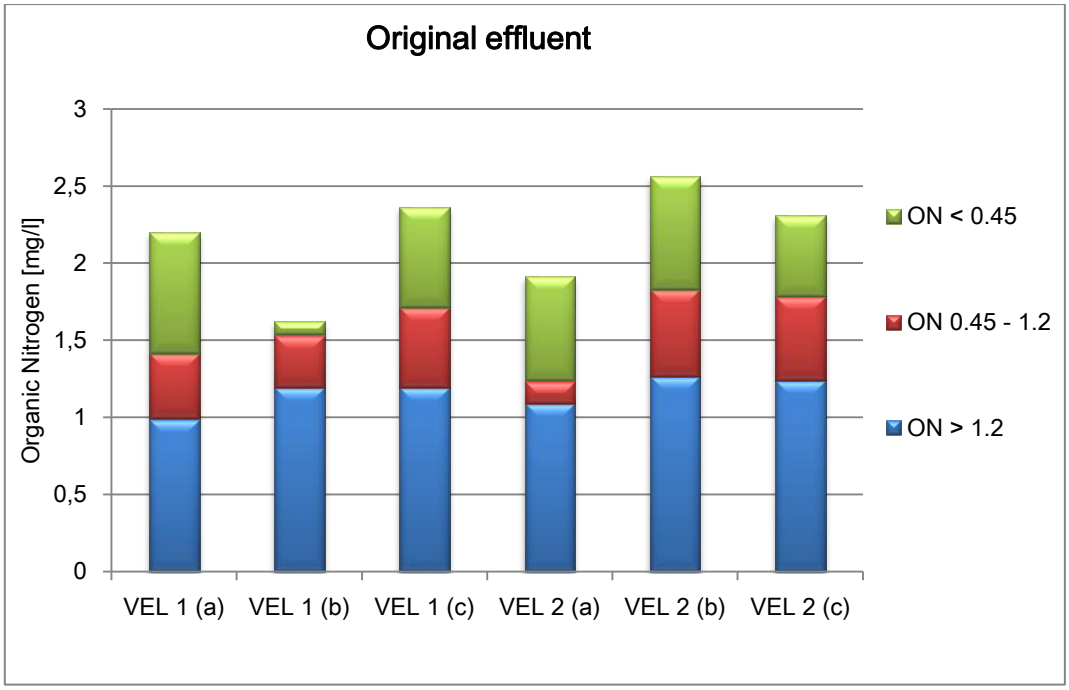

Figure 5.8 – Original effluent organic nitrogen concentrations.

In the effluent it is not found a high fluctuation in $ON_{>1.2}$ concentrations, which was in all the samples less than 1.5 mg/l. As the effluent was collected in the same place and only with 24h in between of each collection, the ON concentrations are similar. The $ON_{0.45\text{-}1.2}$ concentration is in all the samples less than 0.5 mg/l, which was expected, as the effluent is expected to have low colloids due to retention of colloids by sedimentation.

The tests effluent has a majority of $ON_{>1.2}$ N fraction. There was no increase or decrease of the DON concentrations in the different sampling dates. DON slightly varies between 0.5 to 0.8 mg/l excepting the experiment velocity 1 **b** which presents a concentration of 0.1 mg/l of DON, enventually associated to a measurement error.

6. Conclusions

DON as an effluent product in wastewater affects the quality of the receiving water (as in lake, river or sea) in several ways and therefore it is necessary to understand its occurrence and fate.

Questions as, what it is DON and how its fate in wastewater effluents is; become important with the comprehensive assessment of DON impacts, which lead to a recognition of DON importance. Not only for wastewater effluent but as well for water reuse DON gains a significant necessity of research.

The work developed in this thesis uses batch tests in an anoxic reactor, which simulates denitrification process in a biological nutrient removal in a WWTP. The first objective was to evaluate the fate of DON concentrations through process of denitrification with suspended and attached biomass. The second objective was to evaluate the effect of shear stress (through variation of mixing velocity) on DON variation.

The measured concentrations along the denitrification tests showed a low range of DON values between 0.1 mg/l and 1.9 mg/l. Such values can easily be responsible for the impossibility of complying with future discharge limit of 2.2 mg/l of TN.

There is not an explicit tendency for an increase or decrease of DON concentrations, leading to constant DON concentrations. The use of different mixing velocities shows that DON concentrations are not affected.

The use of dry ice to cool down samples allowed the conservation of several samples during a short period of denitrification.

7. Recommendations

In this research DON was measured during denitrification and no tendency for concentration increase or decrease was shown.

In order to better understand DON dynamics it would be usefull to obtain the variation of different fractions of DON during denitrification, such as $DON_{<0.1}$, $DON_{0.1-0.2}$, $DON_{0.2-0.45}$ and $DON_{>0.45}$.

Moreover, the measurement of DON hydrophilic and hydrophobic fractions could show if there is a tendency for bioavailable DON to increase or decrease during denitrification.

The determination of DON and its fractions, during denitrification, along other conventional and non-conventional processes of wastewater treatment, also to better understand DON behaviour.

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Appendixes

Appendix I. Measurments Results

I.I. NO_x concentrations along the denitrification tests

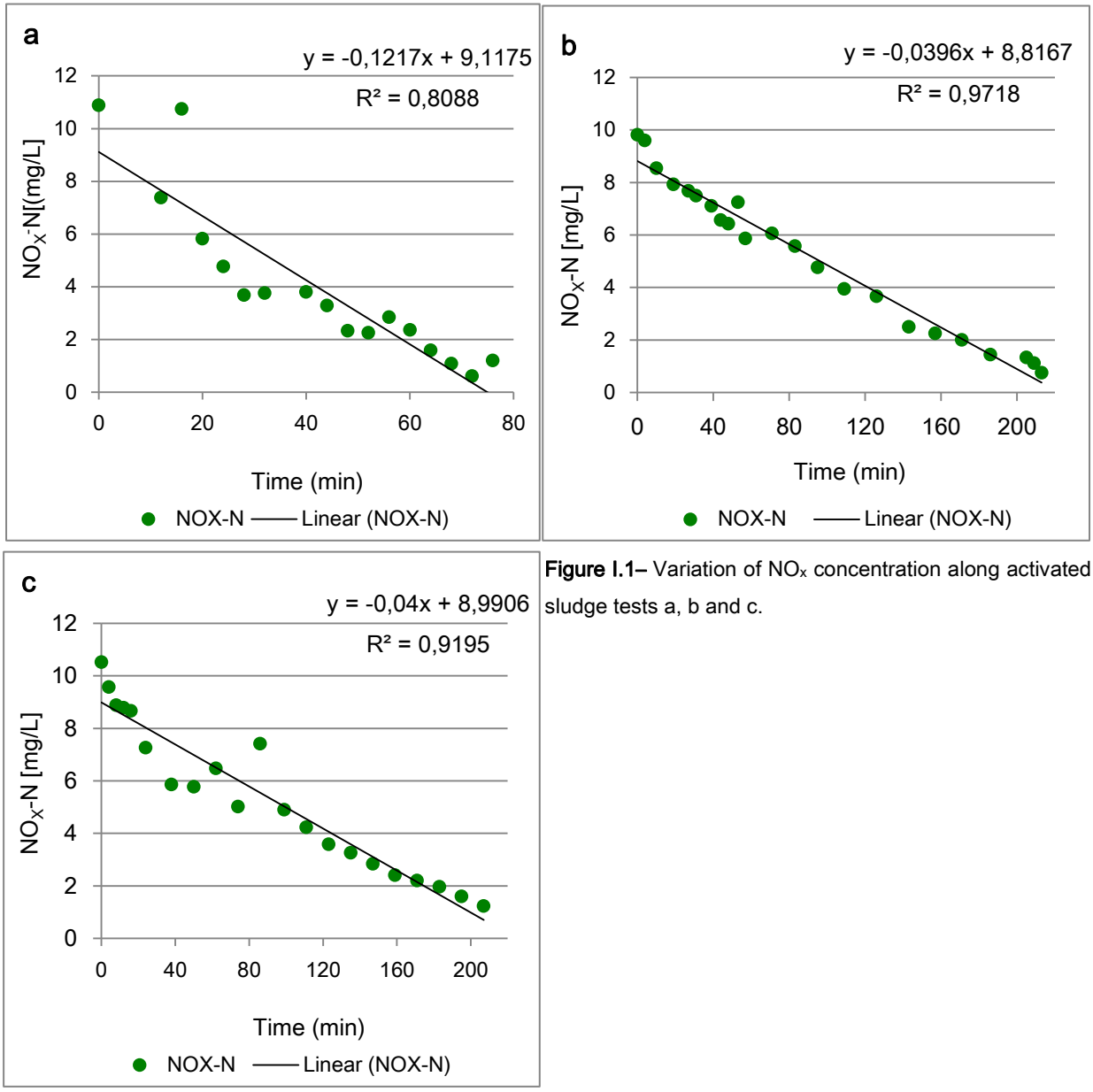


Figure I.1– Variation of NO_x concentration along activated sludge tests a, b and c.

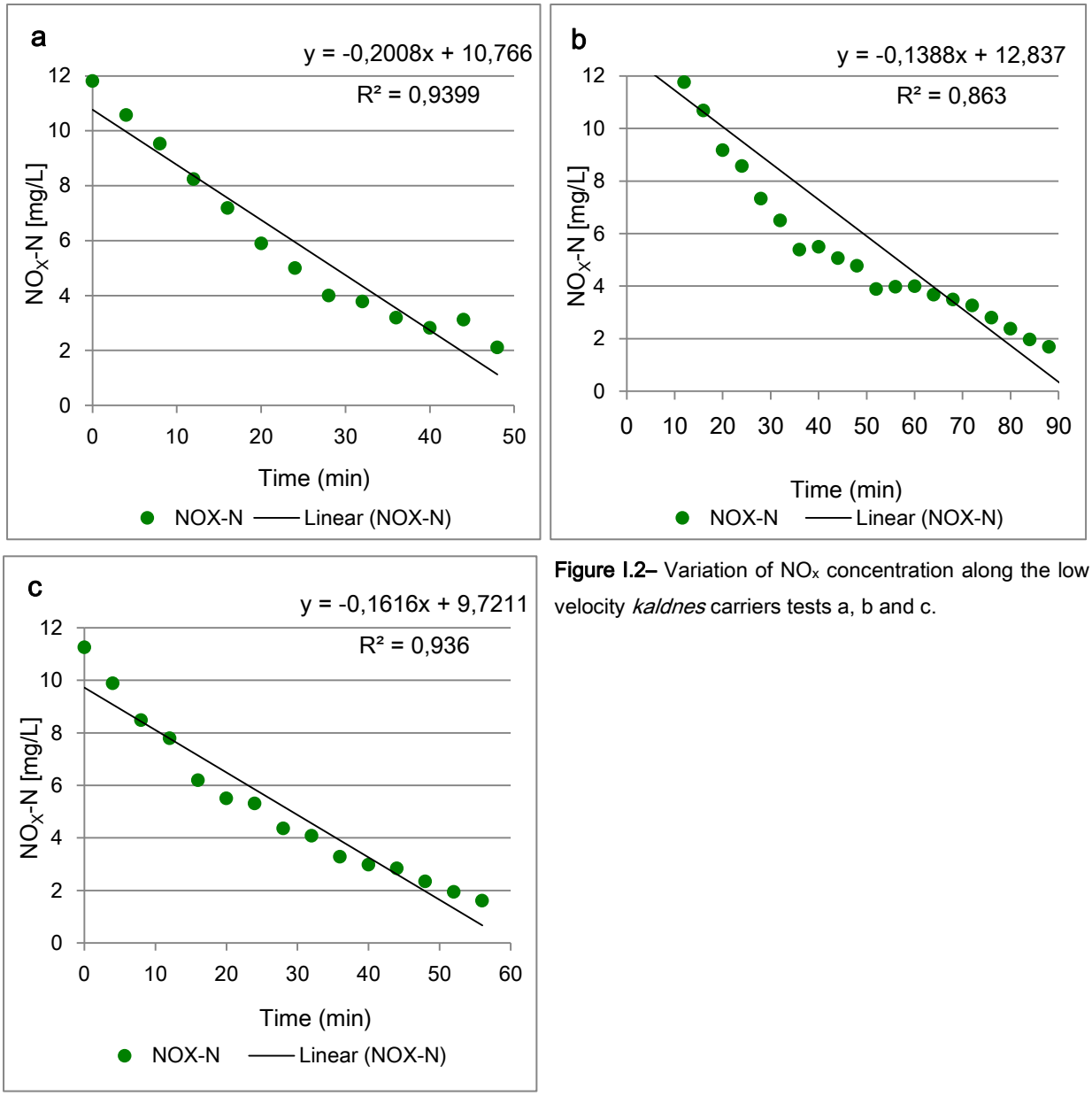
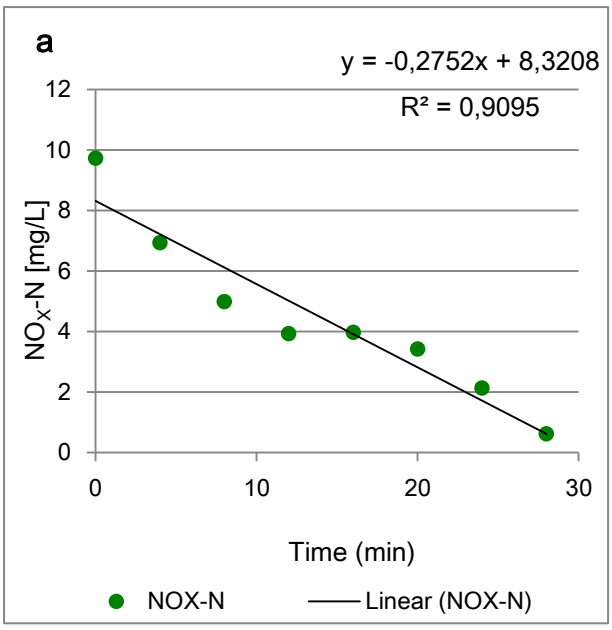
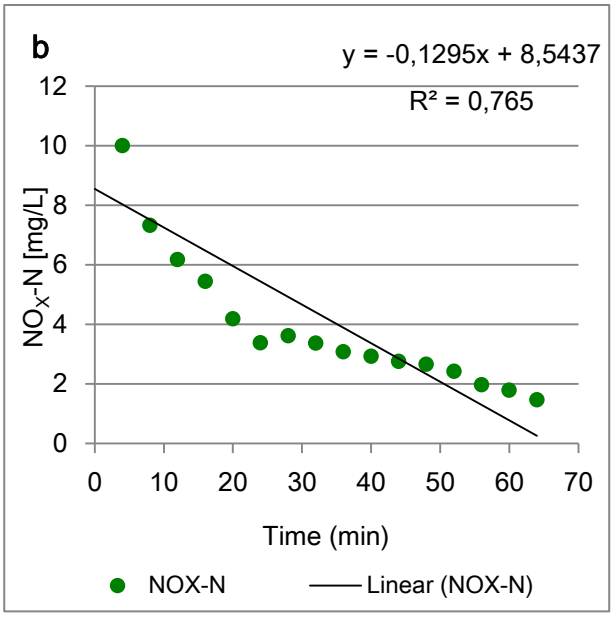


Figure I.2– Variation of NO_x concentration along the low velocity *kaldnes* carriers tests a, b and c.





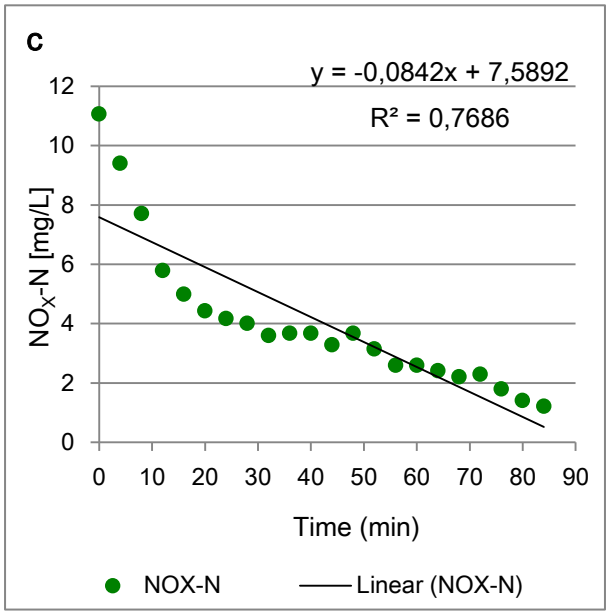


Figure I.3– Variation of NO_x concentration along the high velocity *kaldnes* carriers tests a, b and c.

I.II. Chemical Oxygen Demand along the denitrification tests

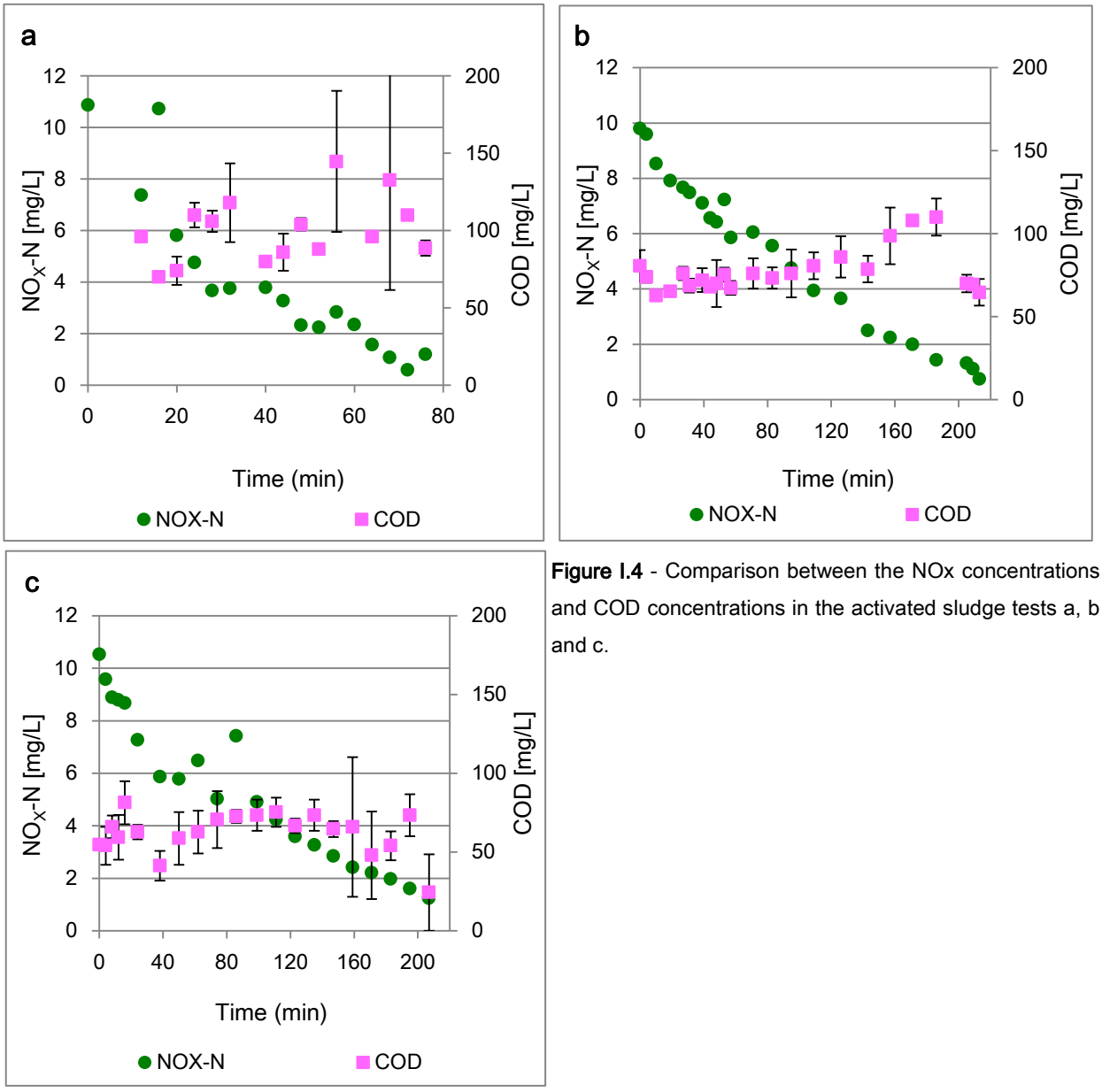


Figure I.4 - Comparison between the NOx concentrations and COD concentrations in the activated sludge tests a, b and c.

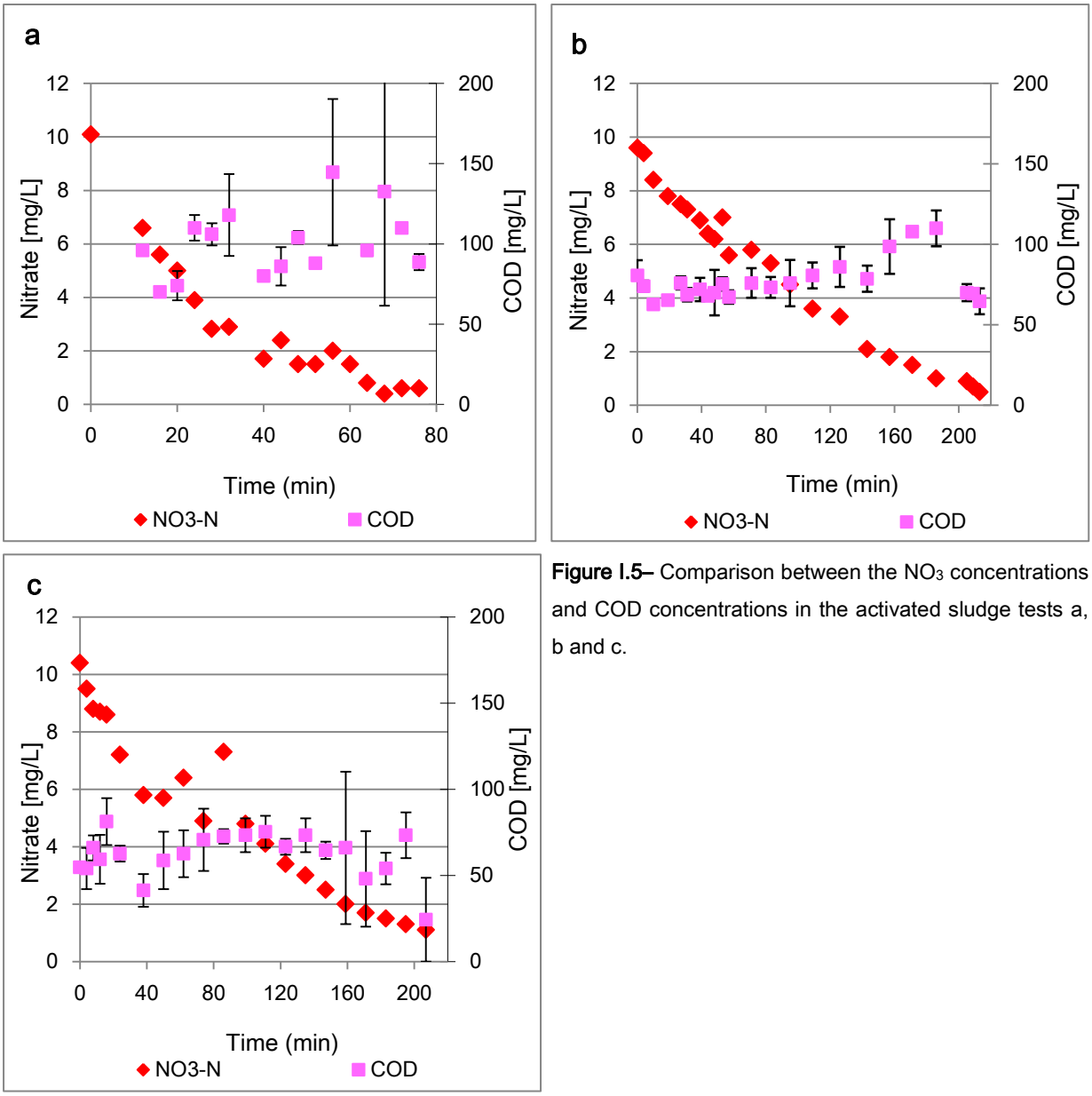
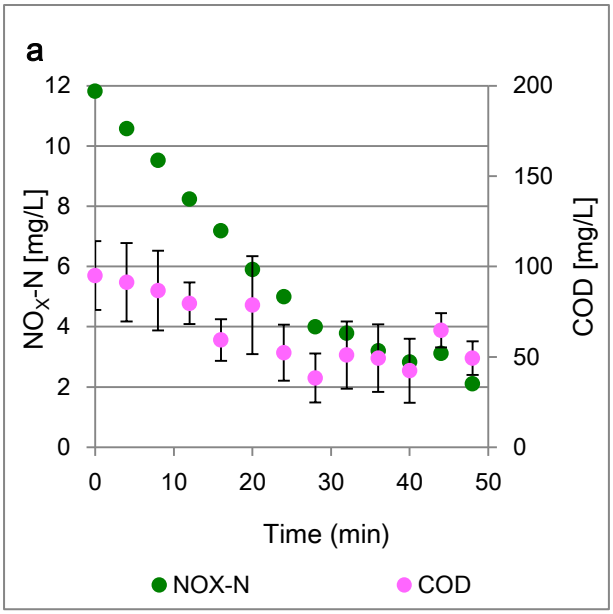
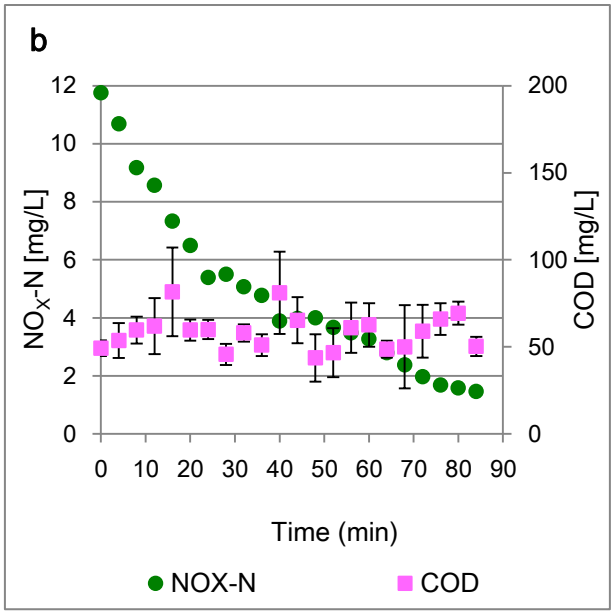


Figure I.5– Comparison between the NO_3 concentrations and COD concentrations in the activated sludge tests a, b and c.





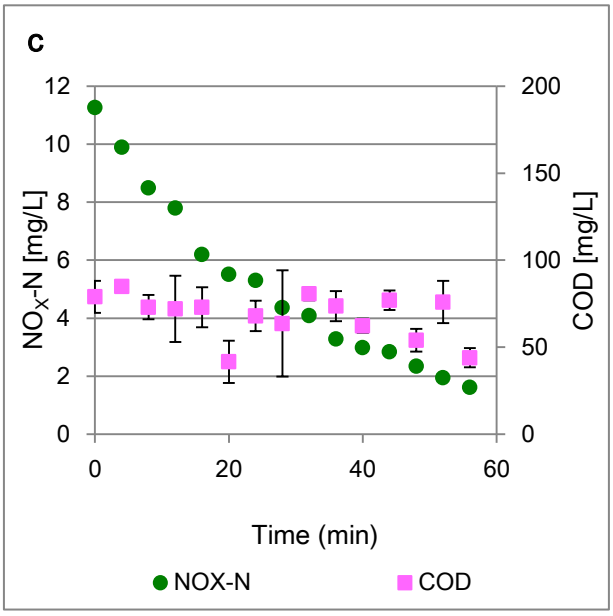
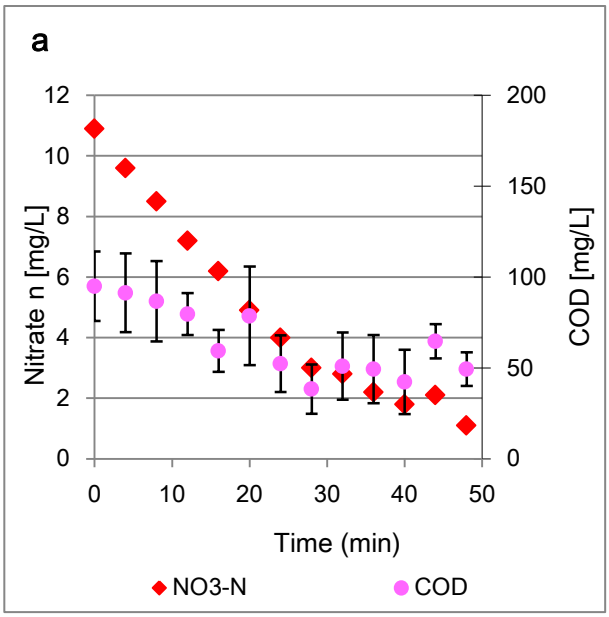
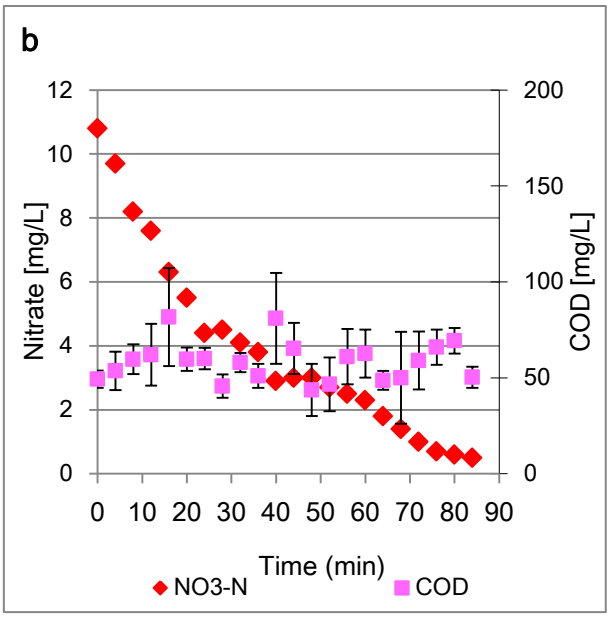


Figure I.6– Comparison between the NO_x concentrations and COD concentrations in the low velocity *kaldnes* carriers tests a, b and c.





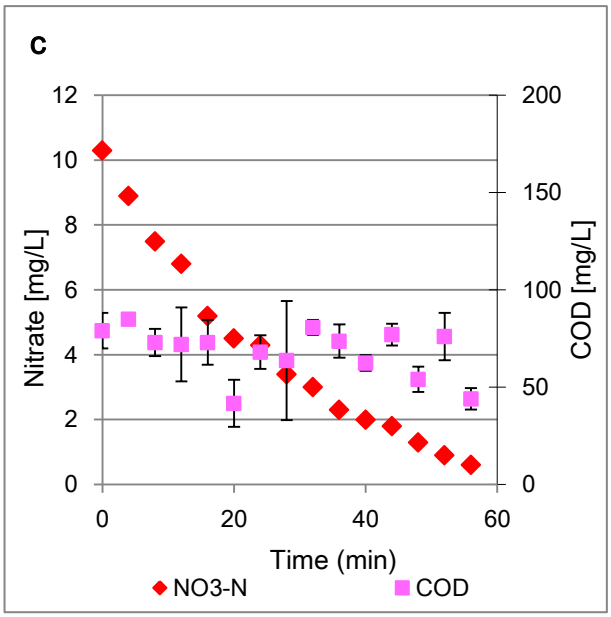
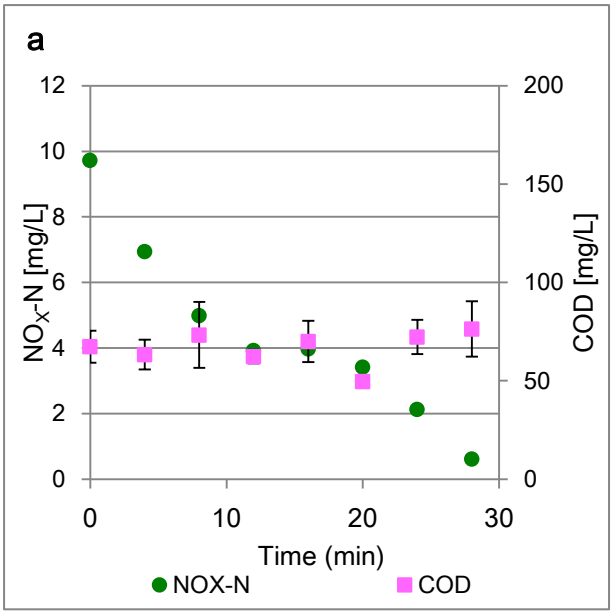
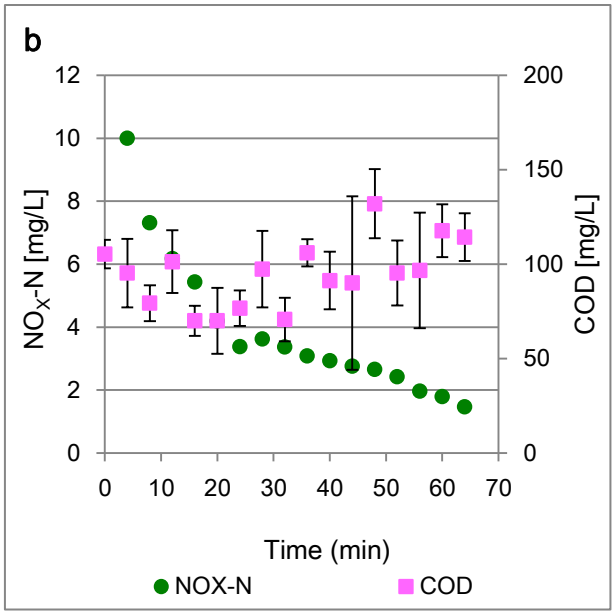


Figure I.7– Comparison between the NO_3 concentrations and COD concentrations in the low velocity *kaldnes* carriers tests a, b and c.





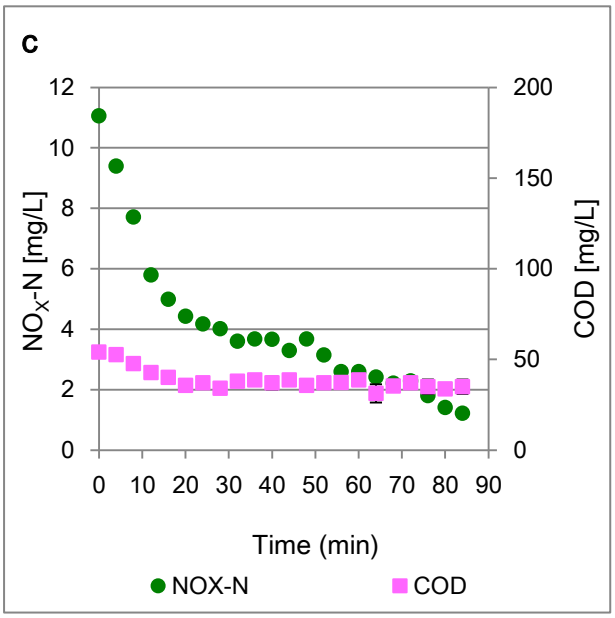


Figure I.8 – Comparison between the NO_x concentrations and COD concentrations in the high velocity *kaldnes* carriers tests a, b and c.

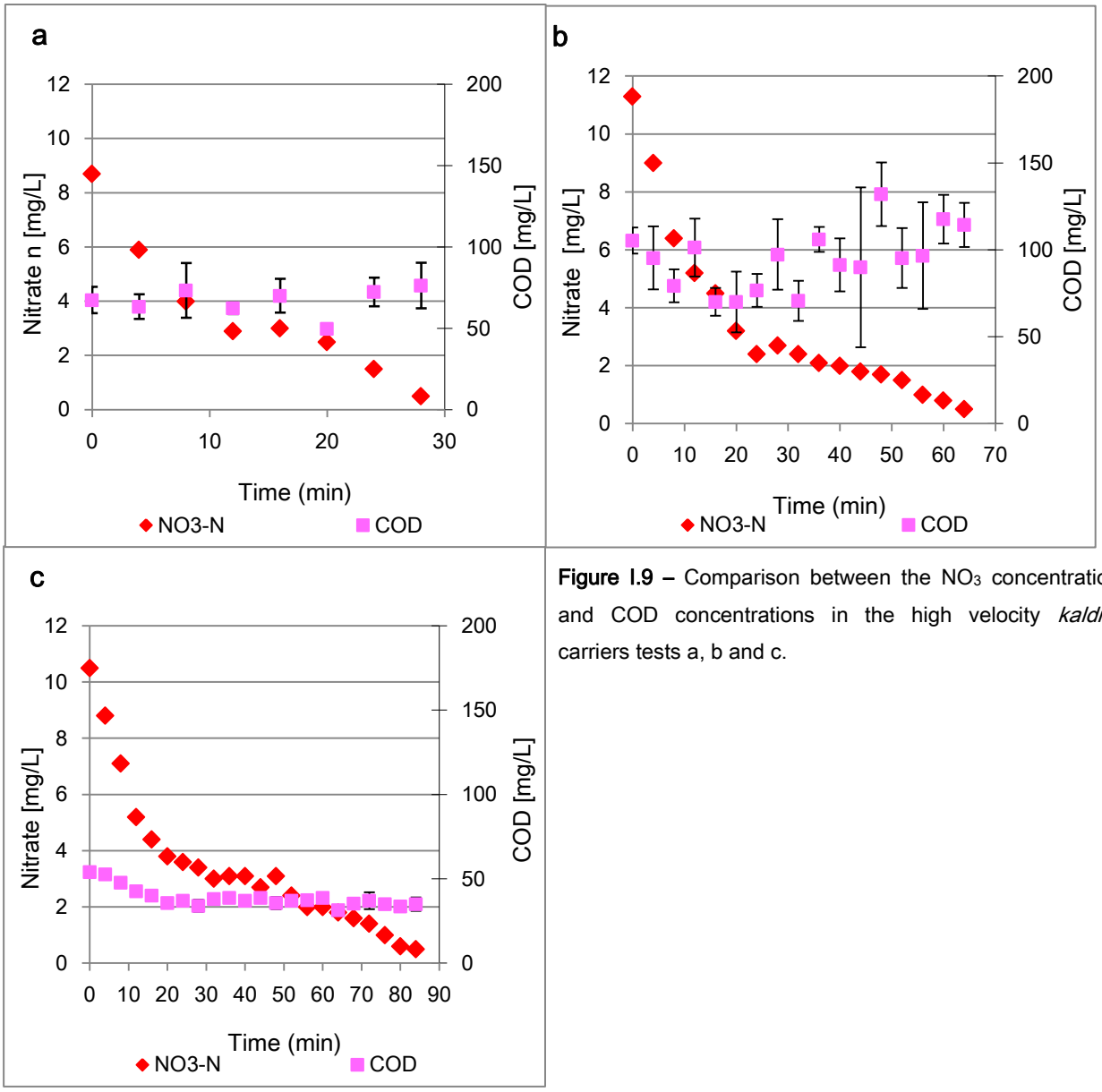


Figure I.9 – Comparison between the NO_3 concentrations and COD concentrations in the high velocity *kaldnes* carriers tests a, b and c.

I.III. Dissolved Organic Carbon along the denitrification tests

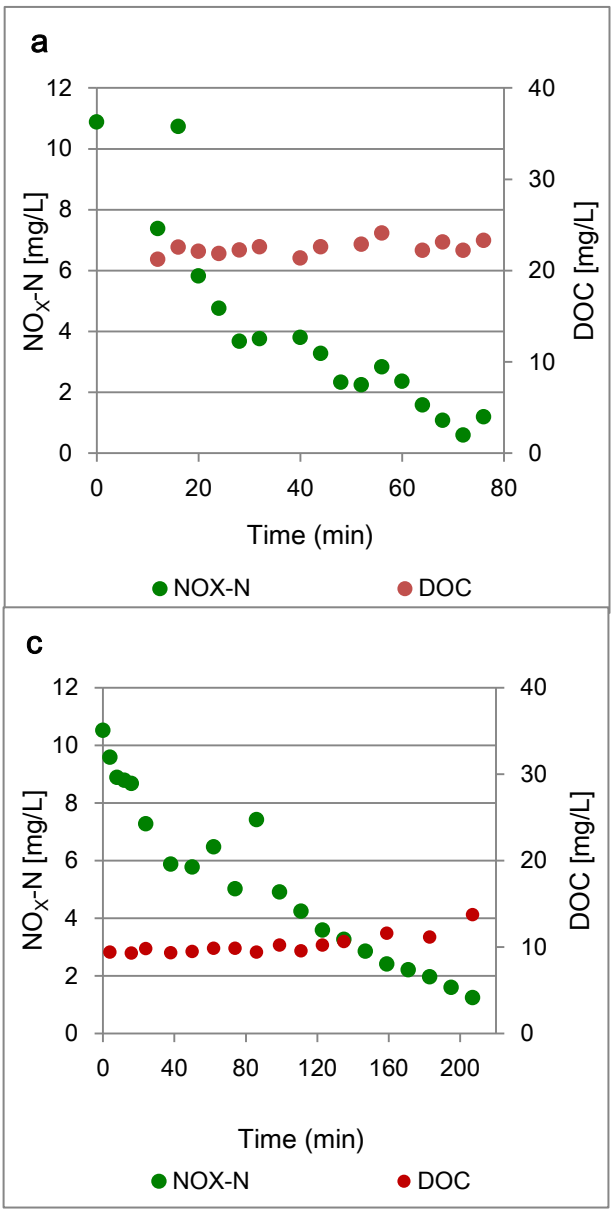


Figure I.10– Comparison between the NO_x concentrations and DOC concentrations in the activated sludge tests a, b and c.

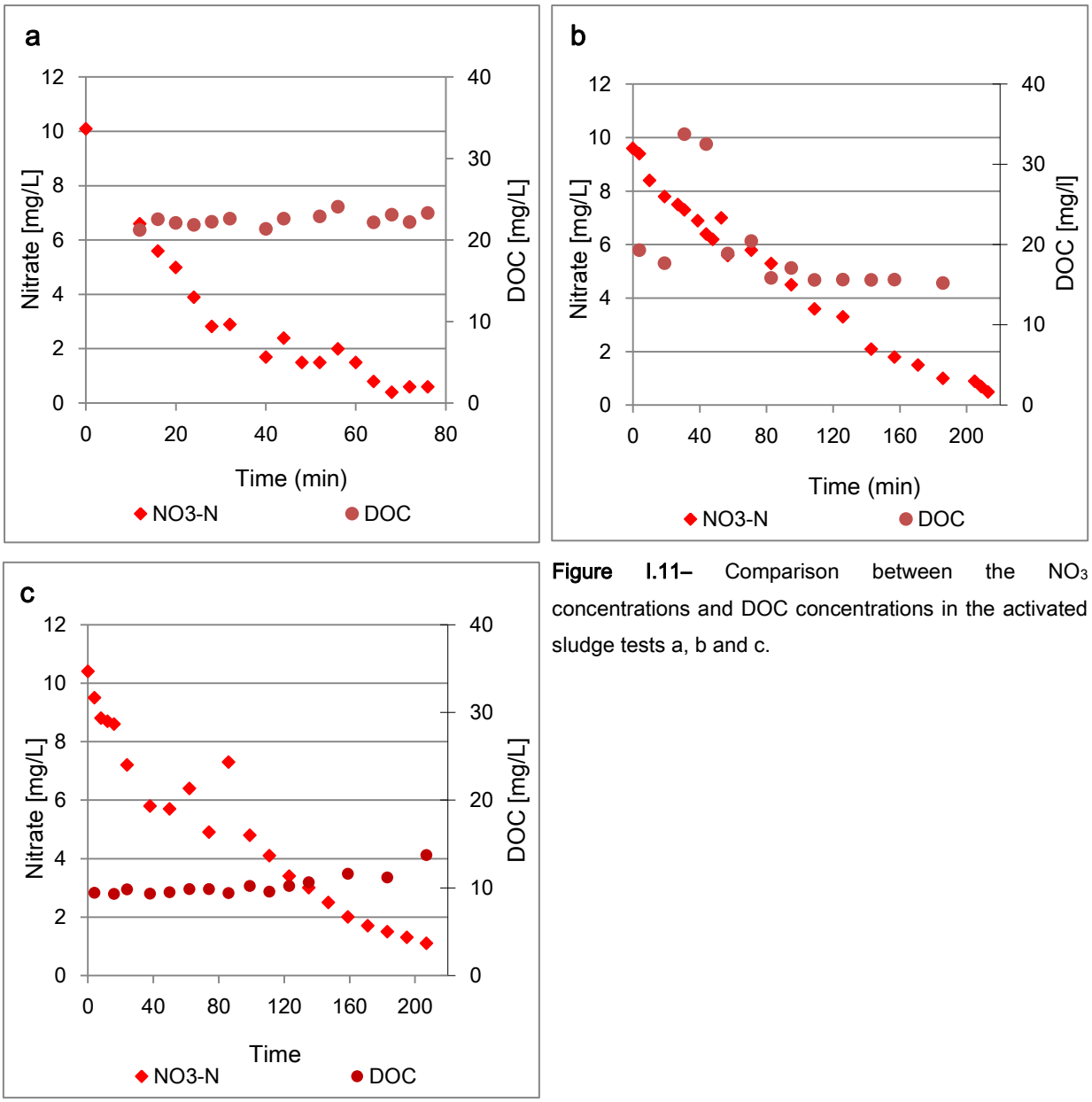


Figure I.11– Comparison between the NO_3 concentrations and DOC concentrations in the activated sludge tests a, b and c.

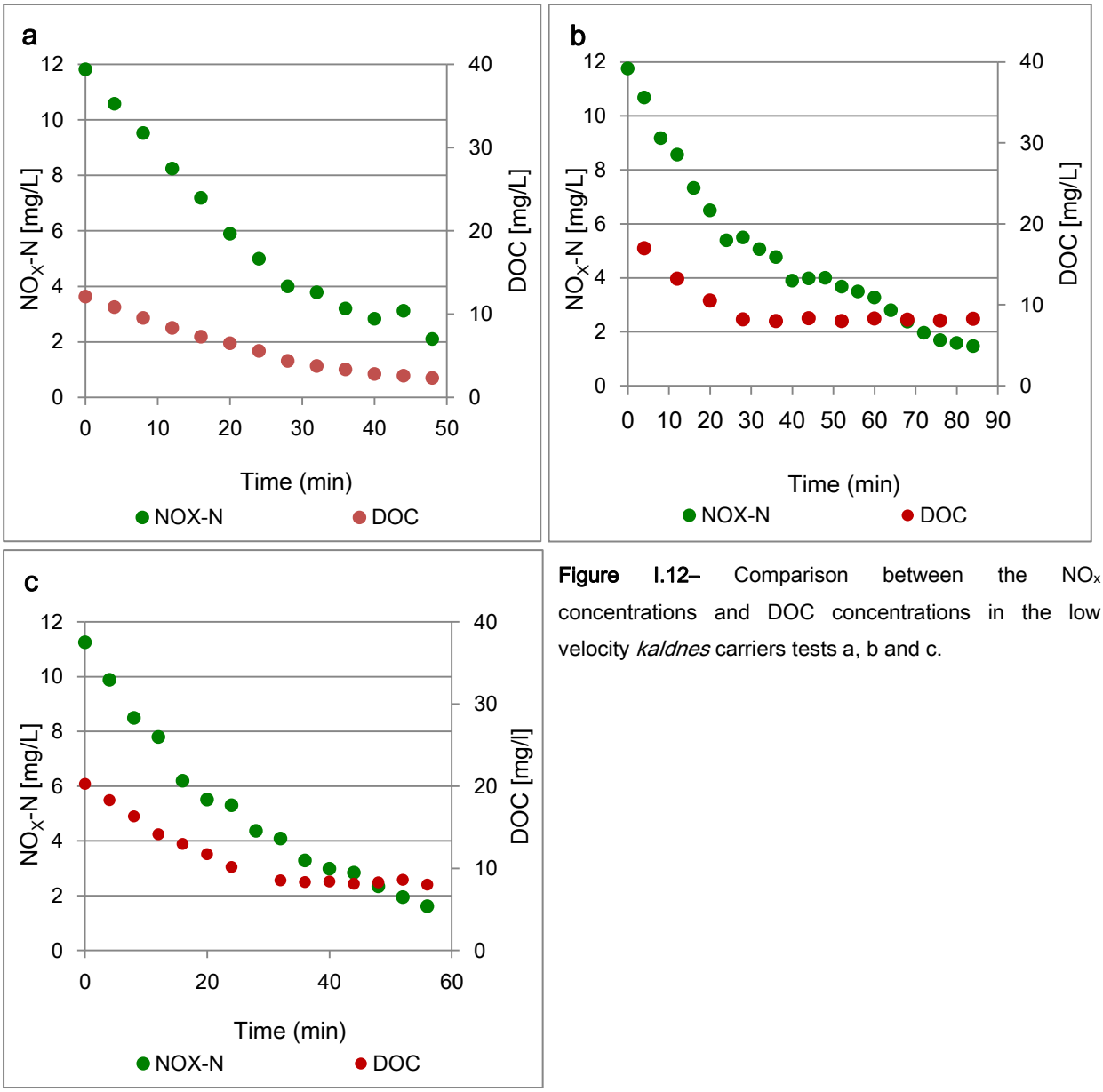
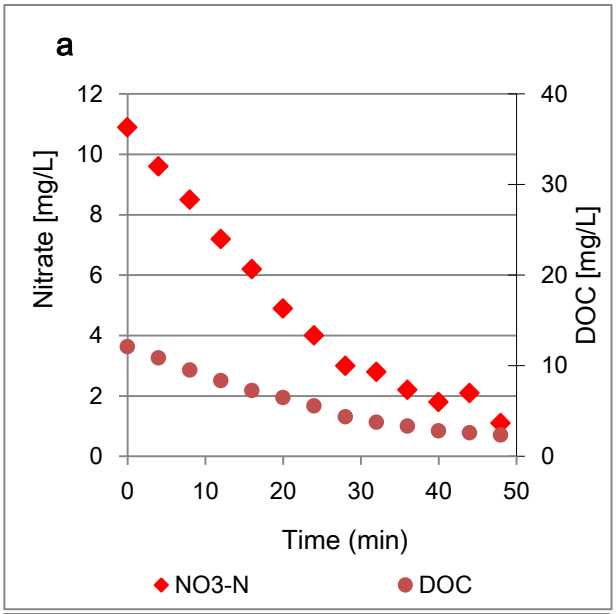
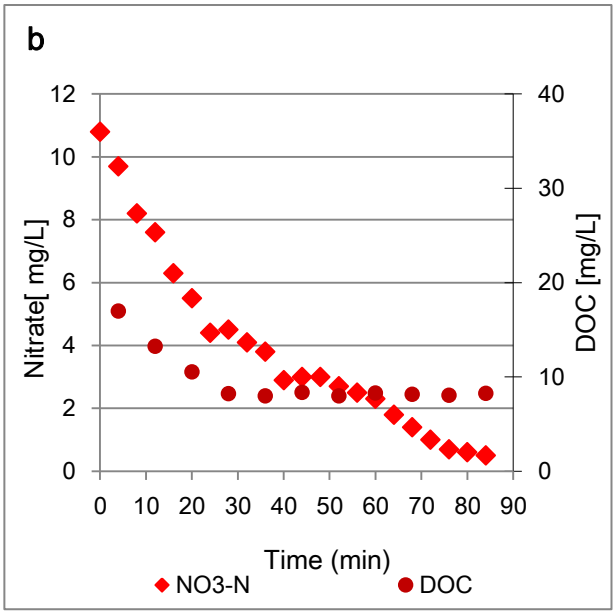


Figure I.12– Comparison between the NO_x concentrations and DOC concentrations in the low velocity *kaldnes* carriers tests a, b and c.





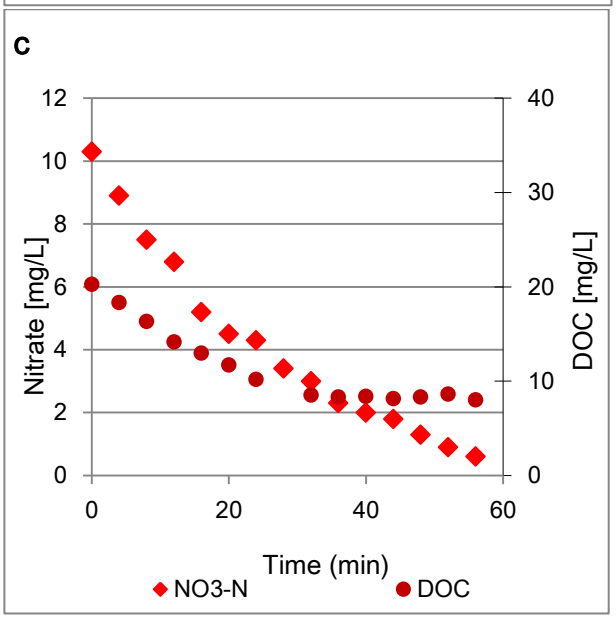


Figure I.13– Comparison between the NO_3 concentrations and DOC concentrations in the low velocity *kaldnes* carriers tests a, b and c.

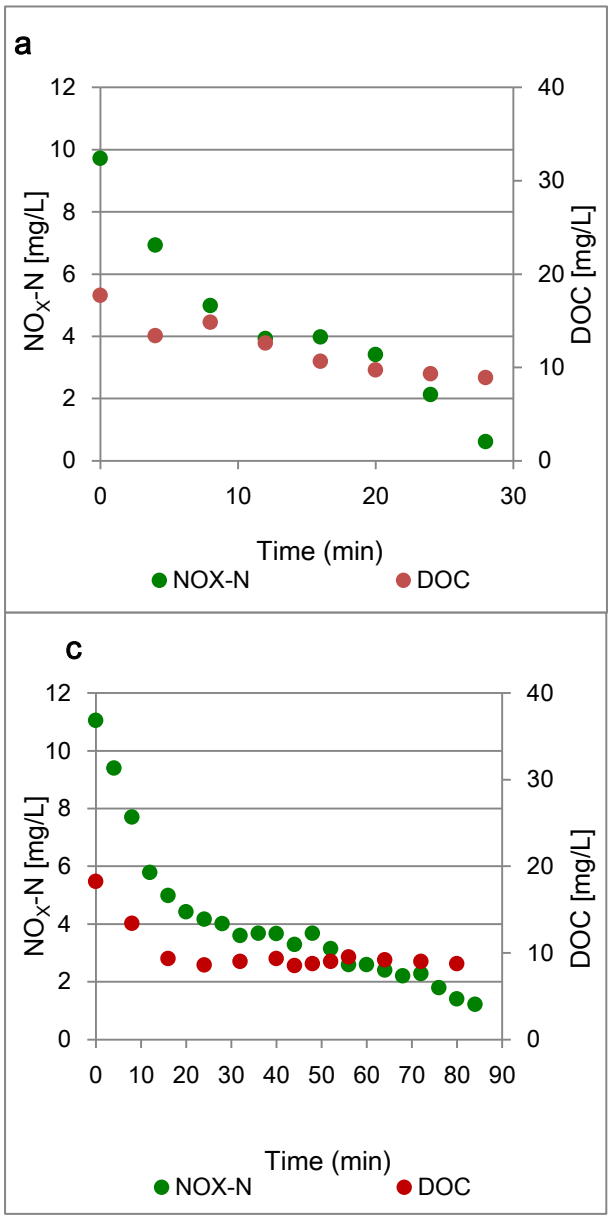
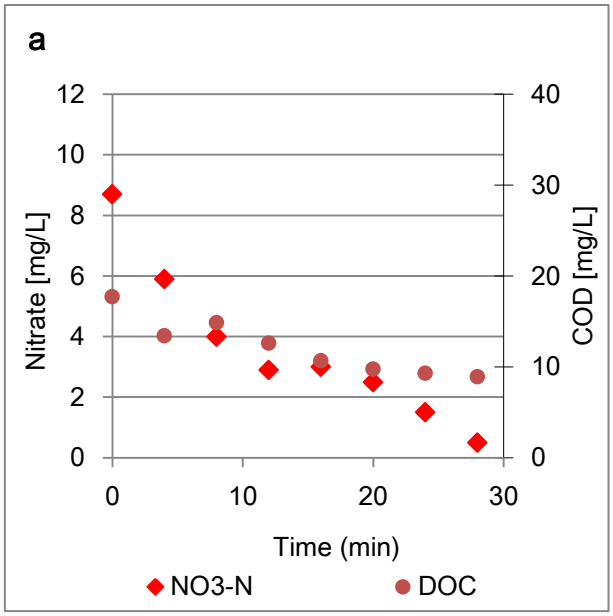
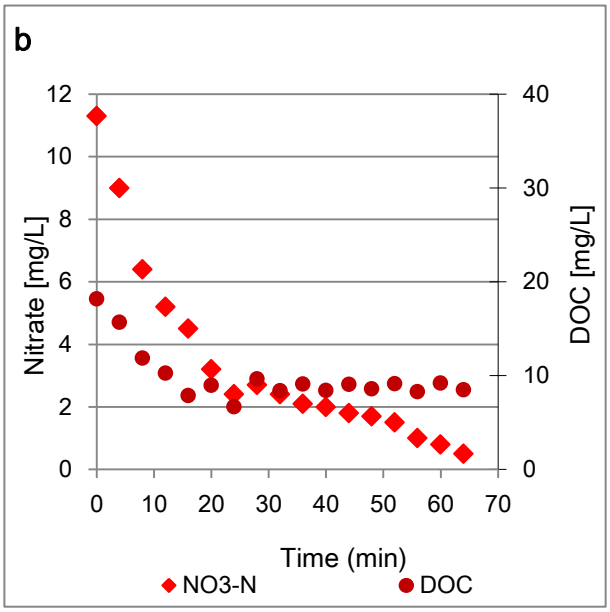


Figure I.14 – Comparison between the NO_x concentrations and DOC concentrations in the high velocity *kaldnes* carriers tests a, b and c.





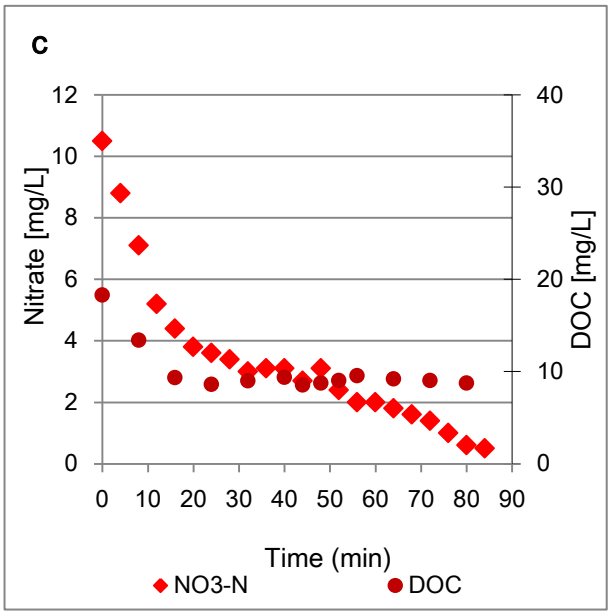


Figure I.15 – Comparison between the NO_3 concentrations and DOC concentrations in the high velocity *kaldnes* carriers tests a, b and c.

I.IV. Dissolved Organic Nitrogen along the denitrification tests

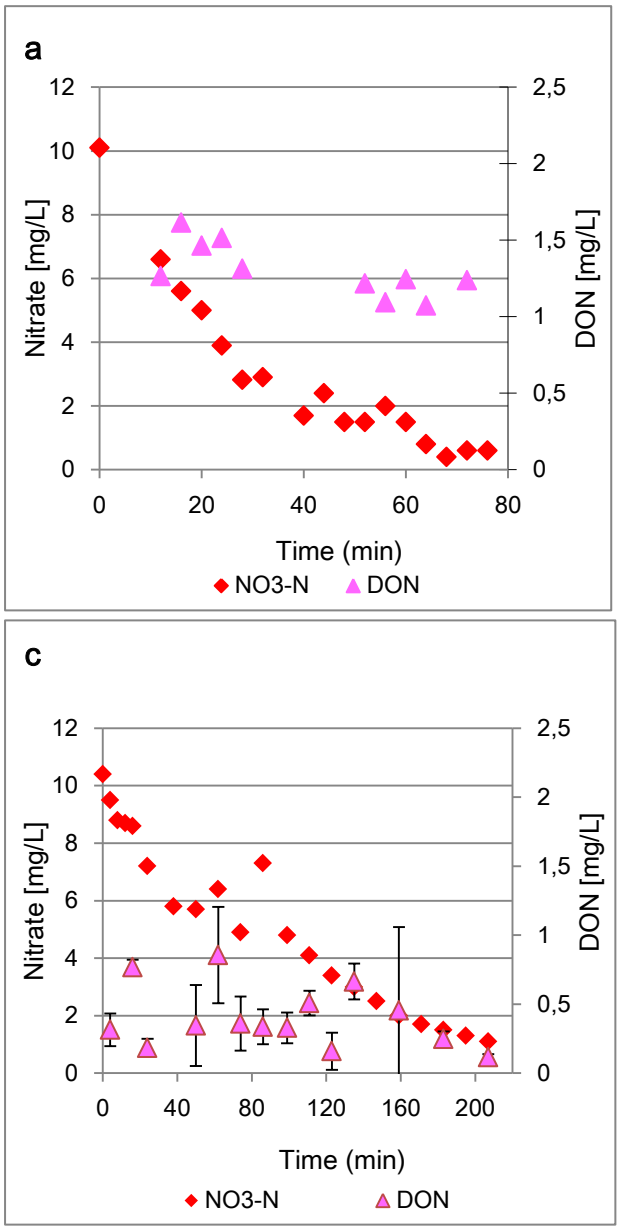
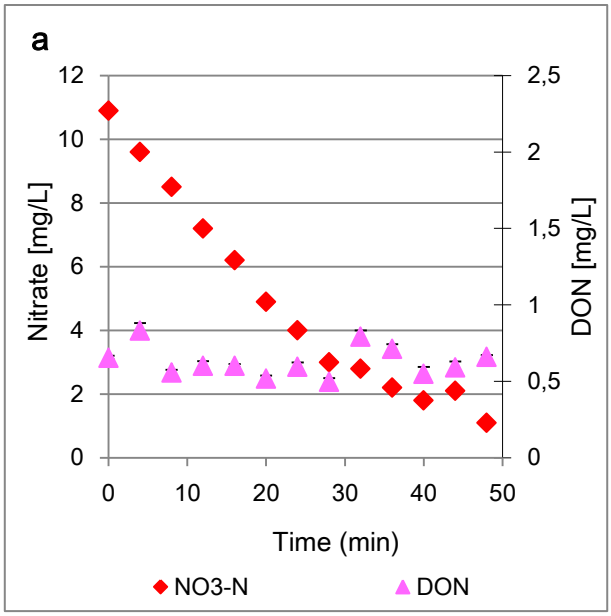
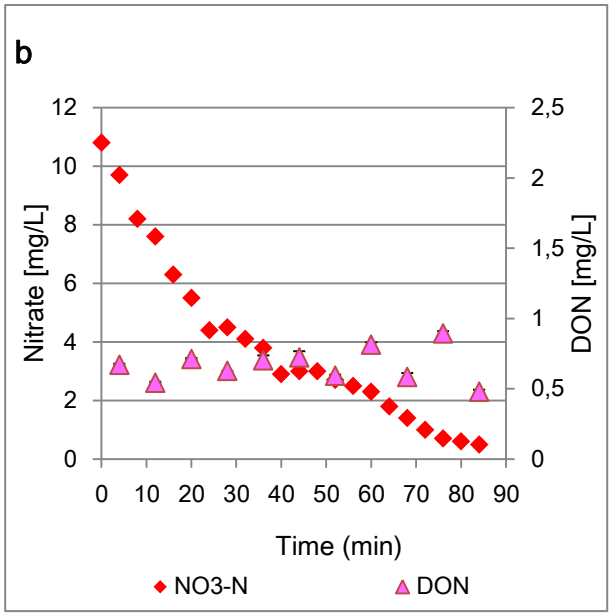


Figure I.16 – Comparison between the NO_3 concentrations and DON concentrations in the activated sludge tests a, b and c.





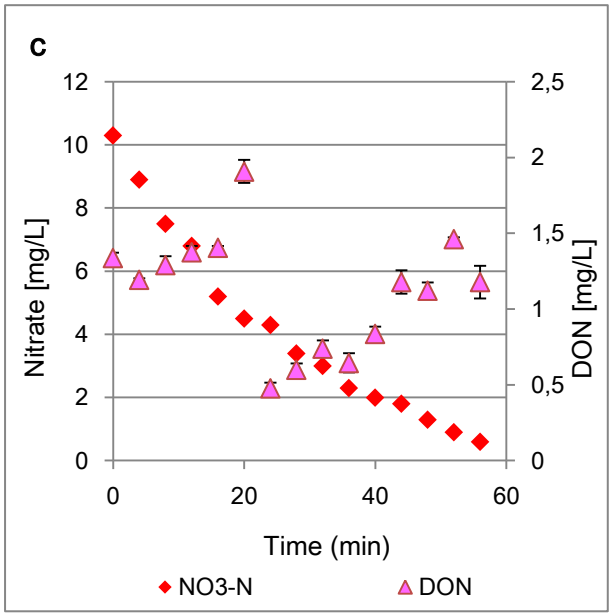


Figure I.17 – Comparison between the NO_3 concentrations and DON concentrations in the low velocity *kaldnes* carriers tests a, b and c.

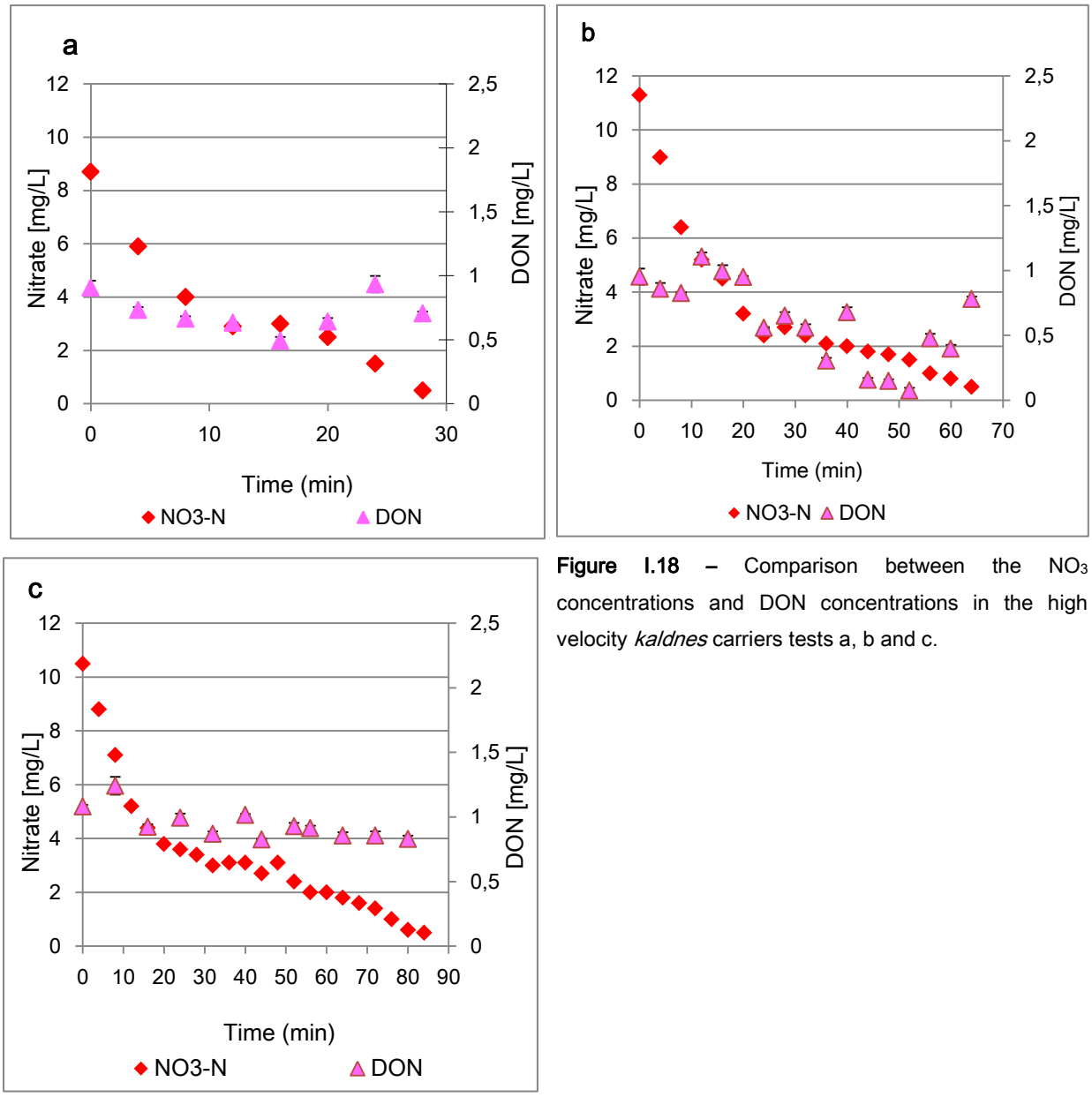


Figure I.18 – Comparison between the NO_3 concentrations and DON concentrations in the high velocity *kaldnes* carriers tests a, b and c.

Appendix II. *Kaldnes* cariers specifications

The biofilm carrier used was the original *kaldnes* carrier (K1) which has been developed in Norway (Hopkins, 2006). The carrier is made of high density polyethylene (density of 0.96 g/cm), is shaped like a cylinder with 7 mm of length and 10 mm of diameter, with a cross inside of the cylinder and fins on the outside (see **Figure II.1**). The specific surface area is given by Metcalf and Eddy (2004) being 500m^2/m^3.

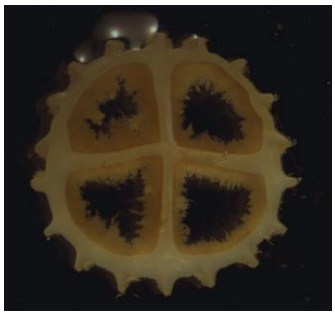


Figure II.1 – *Kaldnes* carrier.
(Adapted from Ødegaard, 1999)

The *kaldnes* characteristics are summarized in the following table 3:

Table II.1 – Characteristics of the *kaldnes* (k1) carrier. (Adapted from Metcalf and Eddy, 2004).

Material	polyethylene
Density (g/cm)	0.95
Specific surface area (m^2/m^3)	500

Appendix III. Preparation of feeding solution

To prepare the synthetic wastewater, two previous solutions were made: an intermediary solution and a trace elements solution. In **Table III.1** the compounds for the intermediary solution and for the trace elements are presented.

Table III.1 – Composition of the synthetic wastewater

Synthetic wastewater (10L)		**Trace elements solutions (1L)**		**Intermediary trace elements solutions (1L)**	
$C_2H_9NaO_5$	34.16g	$CaCl_2{\cdot}2H_2O$	11.396g	H_3BO_3	0.794g
$NaNO_3$	2.91g	$FeCl_3{\cdot}6H_2O$	8g	$ZnCl_2$	0.474g
K_2HPO_4	0.173g	$MgSO_4{\cdot}7H_2O$	5g	$Cucl_2{\cdot}2H_2O$	0.683g
Trace solution	150.6ml	$CoCl_2{\cdot}6H_2O$	2g	$(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$	0.006g
		$NaSiO_3{\cdot}5H_2O$	0.8119g		
		$Al_2(SO_4)_3{\cdot}18H_2O$	0.6026g		
		$MnCl_2{\cdot}4H_2O$	0.0611g		
		Intermediary solution	1ml		

The intermediary solution was made by dissolving the compounds, with their respective weight, in a beaker with Milli-Q water. The solution was transferred for a volumetric flask and made up to 1L.

For the trace elements solution the compounds were dissolved, with their respective weight, in a beaker with demineralized water. The solution was transferred for a volumetric flask and 1ml of the intermediary solution was added. The solution was made up to 1L.

For the synthetic wastewater 150.6 ml of the trace elements solution and the compounds presented in **Table III.1** with their respective weight, were added in 10L of tap water. The solution was mixed and poured directly into the *kaldnes* bucket.