Thermophilic biological nitrogen removal in industrial wastewater treatment

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ABSTRACT

Nitrification is an integral part of biological nitrogen removal processes and usually the limiting step in wastewater treatment systems. Since nitrification is often considered not feasible at temperatures higher than 40^{o}C, warm industrial effluents (with operating temperatures higher than 40^{o}C) need to be cooled down prior to biological treatment, increasing the energy and operating costs of the plants. This study describes the occurrence of thermophilic biological nitrogen removal activity (nitrititation, nitratation and denitrification) at a temperature as high as 50^{o}C in an activated sludge wastewater treatment plant treating wastewater from an oil-refinery. The similar nitrititation and nitritatation rates observed between 35 and 50^{o}C (of 3.3-4.6 mgNH_4^+-N/mgVSS.h and 2.9-4.5 mgNO_2^--N/mgVSS.h, respectively) led to nitrite accumulation. Using a modified 2-step nitrification-2-step denitrification mathematical model extended with the incorporation of double Arrhenius equations, the nitrification (nitrititation and nitratation) and denitrification activities were described including the cease in biomass activity at 55^{o}C. Fluorescence *in situ* Hybridization

(FISH) analyses revealed that *Nitrosomonads* and *Nitrosomonas oligotropha* (known ammonia-oxidizing organisms, AOB) were present in the sludge. However, no nitrite oxidizing organisms (NOB) were observed using the FISH probes applied in this study. These observations suggest that thermophilic biological nitrogen removal can be attained in wastewater treatment systems. It opens the possibilities for the implementation of biological nitrogen removal processes via the NO_2^--N pathway in the main stream, which may further contribute to the optimization and even the re-engineering of wastewater treatment systems that treat warm wastewater streams.

KEYWORDS

Nitrification, higher temperature, Ammonia-Oxidizing Bacteria (AOB), Nitrite-Oxidizing Bacteria (NOB), industrial wastewater.

INTRODUCTION

The biological nitrogen removal (BNR) process via nitrification-denitrification in activated sludge wastewater treatment plants (WWTP) has proven to be an efficient technology to reduce the discharge of nitrogen compounds into surface water bodies. This contributes to keep the concentrations of the nitrogen compounds below toxic levels and avoid eutrophication. Originally developed to treat municipal wastewater in countries with cold and moderate climate (e.g. 10-20°C sewage temperature), the BNR process has also shown to be capable to treat warm industrial wastewater in activated sludge systems (at a temperature as high as 35-40^{o}C) from tanneries and oil-refineries (Moussa *et al.*, 2004; and Pinzon-Pardo *et al.*, 2007). The application of BNR at a higher temperature is usually successful because the optimal temperature for the nitrification process in activated sludge systems is between 35 and 38^{o}C (Buswell *et al*., 1954; Laudelout and van Tichelen, 1960; Henze *et al*., 1995; Hellinga *et*

al., 1998) with a maximum observed cardinal temperature at around 40°C (Henze *et al*., 1995). As a consequence, to avoid treatment process upsets and deterioration, the temperature of industrial effluents (often having temperatures substantially higher than 40°C) is kept below 35-37°C by the application of heat-exchangers and cooling towers. This practice increases the operating costs and is associated with environmental impact due to the higher energy requirements, CO_2 generation and higher water consumption.

As such, to achieve BNR within the thermophilic temperature interval (e.g. between 40 and 60°C; Henze *et al*., 2008) may contribute to significant operational and environmental advantages (such as lesser energy requirements, lower water consumption and lower operating costs). It could also favor the nitritation process and lead to additional potential savings with regard to carbon and aeration requirements by performing nitrogen removal over nitrite (Hellinga *et al*., 1998). Though thermophilic nitrifying activity has been rarely documented in sewage treatment plants (Barrit, 1933; Hellinga *et al*., 1998), it is often observed in soil, leachate and solid waste treatment applications (Stark 1996; Choi and Eum, 2002; Berge *et al*., 2007; Maeda *et al*., 2010). Few recent works have attempted to study the effect of temperature on two-step nitrification by means of laboratory scale bioreactors fed on synthetic wastewater (Guo *et al.*, 2010; Sudarno *et al.*, 2011). However, the understanding of the effect of higher temperature on the performance of nitrifying species is limited.

This study describes the occurrence of BNR activity at a temperature as high as 50°C in an activated sludge plant treating wastewater from an oil refinery. The temperature dependencies of the BNR processes were determined through the execution of short-term (hours) batch experiments. A modified mathematical model was formulated to describe the biological nitrification and denitrification processes observed in the temperature range from 35 to 55°C.

MATERIALS AND METHODS

Batch activity tests

To assess the thermophilic BNR processes and their temperature dependency, a series of short-term (hours) batch activity tests were executed at 35, 40, 45, 50 and 55^{o}C using fresh activated sludge from a WWTP treating oil refinery wastewater (Pinzon-Pardo *et al*., 2007). Currently, the oil refinery WWTP attains satisfactory biological organic matter and nitrogen removal at an average wastewater temperature of 34^{o}C (effluent biochemical oxygen demand, BOD_5, chemical oxygen demand, COD, and total nitrogen concentrations are lower than 25, 125 and 10 mg/L, respectively). The plant consists of three tanks in series; the first two operate in on-off aeration mode with pure oxygen supply for nitrogen removal, whilst methanol is dosed to support denitrification; and the third tank is kept constantly aerobic. Further details concerning the plant process scheme and operating conditions can be found elsewhere (Pinzon-Pardo *et al*., 2007).

For the execution of the short-term batch activity tests, mixed liquor activated sludge were collected at the oil refinery WWTP and stored at 4^{o}C prior to the execution of the tests. The tests were executed within 24h after sludge collection. The batch experiments were designed to assess the nitrification and denitrification activities of the sludge. To study nitrification as a two-step process, 10 mg/L of nitrite (NO_2-N) and 20 mg/L of ammonia (NH_4-N) were consecutively added to estimate the oxidation (activity) rates of nitrite oxidation organisms (NOB) and ammonia oxidizing organisms (AOB), respectively, as described by Moussa *et al*. (2003). Similarly, the denitrification process performed by heterotrophic organisms was assessed through the addition of 10 mg/L of nitrate (NO_3-N) and 10 mg/L of nitrite (NO_2-N) under anoxic conditions. In the denitrification tests, methanol (MeOH) was added as external carbon source to mimic, as much as possible, the operating conditions observed at the full-

scale plant. With the objective of removing any potential residual substrate (carbon or nitrogen compounds) present in the sludge and to acclimatize the sludge samples to the temperature of study, the nitrogen sources and methanol were only added after exposing the sludge for 1 hour to the target temperature. Throughout the activity tests, samples were collected, filtered through 0.45 μm pore size filters, and analyzed to determine the variations of NH_4-N, NO_2-N, NO_3-N and methanol (as COD) concentrations during the execution of the tests.

The experiments were conducted in a double-jacketed fermenter (1 L volume) operated under controlled temperature conditions with the help of a water-bath and a recirculator. In the nitrification tests, continuous aeration using compressed air was provided at a flowrate of 60/L to keep the dissolved oxygen (DO) concentration above 4 mg/L throughout the experiments. Meanwhile, in the denitrification tests, 30 L/h of nitrogen gas were continuously sparged to avoid oxygen intrusion. pH was kept at 7.00 ± 0.10 with the help of a two-way pH controller. Process control was achieved through the operation of a control system (Applikon ADI 1030, associated with accessories, viz. DO probe, pH meter). All analyses, including the determination of the mixed liquor suspended solids (MLSS), mixed liquor volatile suspended solids (MLVSS) and COD were performed in accordance with standard methods (APHA, 1998).

Temperature dependency

In order to describe the temperature dependency of the BNR processess in the thermophilic range of 35 to 55^{o}C (including the collapse in activity at the terminal temperature), an extended Arrhenius equation was applied (Lopez-Vazquez *et al.*, 2007, 2009):

$$r_T = r_{35} \cdot \theta_1^{(T-35)} \left[1 - \theta_2^{(T-T_{MAX})} \right] \quad (1)$$

Where: r_T is the biomass activity at the temperature T; T is the temperature in ^oC; θ_1 is the temperature Arrhenius coefficient θ that describes the biomass activity from 35^oC to the optimal temperature; T_{MAX} is the terminal temperature; and, θ_2 is a second temperature coefficient that describes the declination in activity from the optimal to the terminal temperature.

Modeling the 2-step nitrification-denitrification process

The AMS3 model (Henze *et al.*, 2000), the structure of the nitrification and denitrification model developed by Hellinga *et al.* (1999), and the modified ASM3 model developed by Iacopozzi *et al.* (2007) were adapted to describe the two step nitrification-denitrification processes. However, unlike in ASM3, no storage processes were considered in the anoxic growth kinetics since it has been shown that methanol is not taken up and stored as intracellular polymers but directly consumed instead (Purtschert and Gujer, 1999). Another difference with regard to ASM3 was the inclusion of the maximum growth rate of denitrifiers on methanol (similar to the approach followed by (Purtschert and Gujer, 1999), instead of the use of reduction factors to describe the anoxic activity (on nitrate and nitrite) as a function of the aerobic activity of heterotrophs. Ammonification and hydrolysis were not considered either because it was assumed that in the batch experiments no organic compounds were present in the liquid phase at the beginning of the tests (since the sludge samples had been exposed for 1 hour to the corresponding temperature to be applied in the test). Thus, the model only included those processes that describe the activities of autotrophic and methanol-degrading heterotrophic organisms. Once the 2-step nitrification-denitrification model was defined, the extended Arrhenius equations determined in this study were incorporated into the model to describe the BNR processess observed from 35 to 55^oC.

Microbial identification

Fluorescence *in situ* Hybridization (FISH) analyses were carried out to perform an identification assessment of the nitrifying populations potentially responsible of the observed thermophilic nitrifying activity. Table 1 shows the FISH oligonucleotide probes applied in this study. All FISH analyses were executed in accordance to the hybridization conditions described in the original references (e.g. at the suggested formamide concentrations and using the required competitor probes).

RESULTS

Temperature effects on nitrification and denitrification

To assess the oxidation activities of AOB and NOB, nitrification experiments were carried out on the industrial sludge following the procedure described by Moussa *et al.* (2003). As observed in Figures 1a and 1b, both bacterial groups showed an increase in their specific oxidation rates as the temperature increased from 35 to 50^{o}C, reaching an optimal rate around 50^{o}C followed by a sudden drop in activity and inactivation at 55^{o}C. In particular, the activity of AOB at 35^{o}C (3.3 mgNH_4-N/gVSS.h) was similar to that found by Pinzon-Pardo *et al*. (2007) in a full-scale system operated at the same temperature. As expected (Hellinga *et al.* 1999), the oxidation rates of AOB were higher than those of NOB (i.e. 3.3 mgNH_4-N/gVSS.h and 2.9 mg NO_2-N/gVSS.h at 35^{o}C for AOB and NOB, respectively). However, at 50^{o}C the AOB and NOB oxidation activities were practically identical (4.6mgNH_4-N/gVSS.h *versus* 4.5 mg NO_2-N/gVSS.h at 50^{o}C for AOB and NOB, respectively). The similar AOB and NOB nitrification kinetics led to nitrite accumulation which was somehow expected based on previous studies (*inter alia* Hellinga *et al*., 1998; Kim *et al*., 2008).

Concerning the temperature dependencies, the double Arrhenius expression provided a satisfactory description of the observed activity rates of AOB and NOB from 35 to 55°C (R^2 ~0.99) including the bacterial inactivation at superoptimal temperatures (Figures 1a and 1b).

When compared to nitrification, the temperature dependencies of denitrification using methanol followed a slightly different trend (Figures 1c and 1d). Though the denitrification activities increased from 35 to 40°C (similar to the nitrification rates), neither a well-defined optimal temperature nor a sudden drop in bacterial activities at superoptimal temperatures were observed. Rather, the optimal denitrification rates on nitrite and nitrate were found around 40 and 45°C, followed by a slow bacterial inactivation above 45 and the cease of activity at 55°C (terminal temperature). Due to unknown reasons, the denitrification rate over nitrate at 35°C was considerably higher than the rate determined by Pinzon-Pardo *et al*. (2007) (6.8 *versus* 4.6 $mgNO_3^-$-N/gVSS.h). Nevertheless, the double Arrhenius expressions could also provide a satisfactory description of the temperature dependencies of the denitrification processes on methanol (Figures 1c and 1d). Interestingly, the denitrification rates over nitrite were consistently faster than those over nitrate (40% faster), indicating that the reduction of nitrate to nitrite is the limiting step in denitrification as observed in previous studies (Timmermans and Van Haute, 1983; Nowak *et al*., 1995).

The theoretical COD requirements for nitrate and nitrite removal using methanol are 3.5 $gCOD/gNO_3$-N and 2.2$gCOD/gNO_2$-N, respectively (Mulder *et al*., 2001). In this regard, a stoichiometric ratio of 5.5g COD-MeOH/gNO_3-N was observed in this study that is higher (but comparable) to those reported by Nyberg *et al*. (1992), Purtschert *et al*. (1996), Purtschert and Gujer, 1999; Henze *et al.* (2000) and Pinzon-Pardo *et al*. (2007) of around 4.8-5.2 gCOD-MeOH/gNO_3-N in full-scale applications. Similarly, a ratio of 2.6 gCOD-MeOH/gNO_2-N was

measured for the denitrification process over nitrite that is slightly higher than that observed by Mulder *et al*. (2001). In spite of the higher stoichiometric ratios, they were constant and independent on temperature.

Modeling

The model (presented in Tables S1-S2 in the supplementary material) provided a satisfactory description of the 2-step nitrification (Figure 2), including the nitrite accumulation at the different temperatures of study due to the higher AOB activities. Similarly, the 2-step denitrification activities observed from 35 to 55^{o}C were also well-described (Figure 3), though no nitrite accumulation was noticed due to the higher removal rates of nitrite compared to those of nitrate. The satisfactory description of the 2-step nitrification-denitrification activities was attained through the adjustment of (i) the biomass growth yields on methanol with either nitrate or nitrite as electron acceptors (to 0.48 and 0.33gCOD/gCOD, respectively), (ii) the inclusion of the temperature dependencies of nitrification and denitrification from 35 to 55^{o}C, and (iii) the adjustment of the anoxic decay rate of heterotrophs ($b_{H,N}$) to 0.25d^{-1}. For other parameters, ASM3 default values were used (Henze *et al*. 2000) with the exception of the biomass growth rates of AOB and NOB that were adjusted to 0.15 and 0.04 d^{-1}, respectively (as applied by Hellinga *et al*., 1999).

Microbial identification

The microbial identification via FISH indicated that AOB bacteria from the *Nitrosomonads* (halotolerant and obligatelohalophilic) and *Nitrosomonas oligotropha* lineages were present in the sludge (Figures 4a and 4b). Based on the oligonucleotide probes applied in this study (Table 1), other AOB or NOB were not detected.

DISCUSSION

Higher temperature effects on nitrification and denitrification

Whereas nitrification has been often observed in soils, solid waste treatment and composting applications (Stark 1996; Choi and Eum, 2002; Berge *et al*., 2007; Maeda *et al*. 2010), only a few studies have documented the occurrence of nitrification above 40^{o}C in activated sludge systems (Barrit, 1933; Hellinga *et al*., 1998). Usually, nitrification fails at temperatures higher than 40^{o}C (Sudarno *et al.*, 2011). Nevertheless, in those studies where nitrification was observed above 40^{o}C, the optimal temperature was found around 37-40^{o}C (Stark, 1996; Hellinga *et al*., 1998). Conversely, in this study 50^{o}C was the optimal temperature for nitrification. Despite that the higher wastewater operating temperature at the refinery (34^{o}C) could be assumed to be the main factor that leads to the selection of thermophilic organisms, certainly there appear to be other operating and environmental factors. In an attempt to explore the key factors that lead to the selection of thermophilic nitrifying and denitrifying bacteria in industrial wastewater treatment plants, Chikamba (2009) executed a similar set of experiments on different plants (involving another oil-refinery, a food and beverage industry, a fermentation industry and another tannery) operated at between 30 and 35^{o}C, and one municipal plant operated at 15-20^{o}C. However, all failed to show bacterial activity above 40^{o}C. The use of pure oxygen in the plant described in this study appears to be the main difference compared to the plants studied by Chikamba (2009). In a study performed for 2 years in a lab-scale system fed with 130 mg NH_4-N/L, Hellinga *et al*. (1998) observed ammonium oxidation activity up to 55^{o}C with an optimal temperature around 40^{o}C. They reported that the selection pressure created by the applied operating conditions (such as high N-concentrations, high temperature and short SRT) led to the selection of organisms with a lower affinity for substrates (e.g. higher half-saturation constants for substrates such as ammonia and oxygen). Thus, higher nitrogen concentrations can be hypothesized to be

another relevant factor (in addition to the higher temperature), implying that higher influent nitrogen concentrations (potentially combined with the return of high-strength N-containing flows from the sludge handling facilities) contribute to the selection of thermophilic nitrifying bacteria. In this regard, the oil-refinery wastewater treatment plant where the sludge was collected contained around 205 mg/L of Total Kjeldhal Nitrogen (TKN) and 93 mgNH_4-N/L (Pinzon-Pardo *et al*., 2007), which could have favored the growth of thermophilic organisms. Nevertheless, it cannot be discarded that the use or a combined effect of different factors such as higher temperature, higher nitrogen concentrations, use of pure oxygen rather than air (which can increase the oxygen transfer efficiency), salinity levels, reactor configurations, internal recycle of high strength nitrogen flows can potentially lead to the selection of thermophilic nitrifying organisms in full-scale activated sludge systems.

Interestingly, the denitrification rates over nitrite were consistently higher than those over nitrate indicating that the reduction of nitrate to nitrite is the limiting step in denitrification as also observed in previous studies (Timmermans and Van Haute, 1983; Nowak *et al*., 1995). Two potential explanations could be (i) the potential inhibition of nitrate reduction due to the presence of nitrite (Timmermans and Van Haute, 1983) or (ii) that nitrite is more reduced than nitrate requiring lesser COD (3.5 gCOD/gNO_3-N and 2.2gCOD/gNO_2-N) (Mulder *et al*., 2001).

As expected, above 30^{o}C the activity of AOB was higher than that of NOB, favoring the accumulation of nitrite. Also, the denitrification activity over nitrite proceeded twice as fast as the denitrification over nitrate (also requiring about 40% less COD). These conditions suggest that it can be potentially feasible to achieve BNR via nitritation and denitritation, contributing to reduce the operational costs of the plants in terms of aeration and external carbon source

dosage. Furthermore, it must be noticed that, if the influent wastewater does not contain toxic or inhibitory compounds to Anammox bacteria, the environmental and operating conditions of this plant (e.g. higher temperature, high N concentrations and, therefore, high potential for partial nitrification) make it a suitable candidate for the implementation of the Anammox process in the main-stream treatment line (Kartal *et al*., 2010). The latter may further reduce the environmental impact and operational costs (due to the lower gas emissions and because methanol would not be longer required for denitrification). Further research is needed to elucidate the factors that influence the occurrence of thermophilic nitrifying organisms and event to explore the potential implementation of the Anammox process in the main-stream ine of such industrial wastewater treatment plants. This can potentially lead to the re-design of current industrial activated sludge systems that deal with high N-concentration wastewater streams.

Temperature dependencies

The Arrhenius temperature coefficients of the nitrifying organisms observed in this study (Table 2) were considerably lower than those reported previously (e.g. 1.02 and 1.03 *versus* 1.12), indicating a low sensitivity or temperature dependence from 35 to 55^{o}C. Similarly, the temperature coefficients for denitrification were in the low range of those reported elsewhere for similar cultures (Table 2). Accordingly, previous studies had already suggested the need to develop and apply different models to describe the activity of organisms at temperatures higher than 25^{o}C (Wett *et al*., 2011). The mains reason being that ASM1 and ASM3 are only limited to a temperature range between 10 and 25^{o}C (Henze *et al*., 2000), and that the use of the temperature coefficients proposed in those models overestimate the activity of nitrifying bacteria above 25^{o}C (Wett *et al*., 2011). Thus, the double Arrhenius expressions developed in this study proved capable to describe the nitrification and denitrification activities from 35 to

50°C including their inactivation (Figure 1). Though attempts were made to expand the applicability to a broader temperature range, the expressions developed in this study were not suitable to describe the activities out of the temperature range studied in this research (e.g. below 35°C or above 50°C) (data not shown). Trying to find a unified expression, another attempt was made to use the quadratic temperature dependency equation developed by Wett *et al*. (2011) for nitrifying organisms for a temperature interval between10 and 50°C. However, a satisfactory description was not obtained. Possibly, the nitrifying activities observed in the different studies are a reflection of the occurrence of different nitrifying communities that have different temperature dependencies. This implies that the expressions are case-sensitive and likely dependent on local conditions and on the occurrence of the nitrifying organisms.

Modelling

After the adjustment of the biomass growth yields of denitrifying organisms, the incorporation of the double Arrhenius temperature expressions and fixing the anoxic decay rate of heterotrophs, the modified ASM3 model developed in this study proved capable to correctly predict the activity of the nitrifying and denitrifying organisms from 35 to 55°C (Figures 2 and 3). The anoxic growth (denitrification) on storage polymers (as described in ASM3) was not included since it has been shown that methanol is not converted into storage polymers but consumed directly (Purtschert and Gujer, 1999). This occurs likely due to the involvement of a specific group of methanol-degrading organisms (*Hyphomicrobium sp*.), rather than to the presence of ordinary heterotrophs, as postulated by Timmermans and van Haute (1983). The satisfactory description of the biomass activities supports this approach (Figure 3).

The anoxic decay rate of ordinary heterotrophs (b_{HN}) was fixed to 0.25 d^{-1} instead of keeping the values given by the Arrhenius temperature expressions of ASM3 because they tended to

considerably overestimate the denitrification processes over nitrite and nitrate as temperature increased (data not shown). As observed in Figure 3e for the denitrification batch experiments executed at 55^{o}C, the model correctly predicts no consumption of methanol. However, since the anoxic decay process takes place continuously the model still predicts the consumption of nitrite and nitrate, which was more pronounced before fixing the anoxic decay rate at 0.25d^{-1}. Salem *et al.* (2006) also inferred that the temperature coefficients for autotrophic decay in ASM overestimate the decay at higher temperature. Due to the lack of information concerning the temperature effects on the anoxic decay rate, the constant value of 0.25 d^{-1} provided a satisfactory description. Nevertheless, further research is needed to determine the temperature dependency of this process.

In this manuscript, an extended ASM3 model describing nitrification as a two-step process and the simultaneous denitrification on nitrite and nitrate was presented. This enabled the model to correctly describe nitrite accumulation during nitrification and the facultative denitrification on nitrite and nitrate using methanol as external carbon source. The incorporation of double Arrhenius expressions to describe nitrification and denitrification enabled to extend the application of the model to a temperature beyond that considered in ASM1 and ASM3. As such, this model has the potential to be used as a reliable tool to assess and possibly improve and optimize the operation of wastewater treatment plants performing biological nitrogen removal at temperatures higher than 35^{o}C.

Microbial communities

Interestingly, only two groups of AOB (*Nitrosomonads* and *Nitrosomonas oligotropha*) were identified using FISH (Figures 4a and 4b). However, no NOB were detected. In other wastewater treatment applications, AOB such as *Nitrosomonas oligotropha* have also been

observed as well as *Nitrosomonas nitrosa-like* and *Nitrosomonas nitrosa* at temperatures higher than 30°C but lower than 45°C (Avrahami *et al*. 2011; Shore *et al*., 2012). Nevertheless, other types of AOB (*Nitrosomonas europaea/eutropha* clusters) have also been identified at higher temperature in manure composting applications at 70°C at high N-concentrations (of around 2000mg NH_4-N/gTSS) (Yamamoto *et al*., 2010). As discussed previously, the latter is in accordance to previous observations regarding the favourable effect of high N-concentrations on the occurrence of thermophilic nitrifying organisms.

Concerning NOB, *Nitrospira spp.* has been observed in other studies between 30 and 45°C (Shore *et al*. 2012), but they were not detected in this study either. As such, a broader molecular study is needed to identify the nitrifying organisms responsible for thermophilic nitrification in this and other industrial wastewater treatment plants operated under similar conditions (e.g. high temperature with high N-concentrations, using pure oxygen as electron acceptor and methanol as electron donor).

Recent studies (Gilch, 2009) performed on *Nitrosomonas europaea* revealed that on the gene level the optimum temperature for the reaction of hydroxylamin conversion by ammonia monooxygenase lies around 45 to 50°C. This suggests that the environmental and operating conditions applied in engineered systems (such as sewage treatment plants) are usually not optimal and therefore might limit the biological ammonium and nitrite oxidation processes. Aspects such as the ammonia and nitrite concentrations (and even the reactor configurations the recirculation of internal and reject flows) and the dissolved oxygen concentration need to be correctly taken into account since they may play an important role affecting the occurrence of the microbial communities involved in BNR at full-scale (industrial) activated sludge wastewater treatment plants.

These findings can be of major importance to contribute to reduce the operational costs of wastewater treatment plants that treat warm industrial wastewaters. Assuming that in the oil-refinery industry the initial wastewater temperature is around 65^{o}C (personal communication with plant practitioners), the thermophilic BNR would allow to reducing the temperature in only 15^{o}C (to 50^{o}C) instead of having to reduce it in about 30^{o}C (to around 35^{o}C). This implies a reduction of up to 50% in the cooling capacity that can help to significantly reduce the operational costs and environmental impact of several industries (not only from the oil-refinery industry but also from the food industry, tanneries, among others).

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LIST OF FIGURES

Figure 1 - Temperature effects from 35 to 55°C on the activity of (a) ammonia-oxidizing bacteria (AOB), (b) nitrite-oxidizing bacyeria (NOB), (c) denitrification over nitrate and (d) denitrification over nitrite.

Figure 2 - Model simulations of the nitrifying activities of ammonia-oxidizing organisms and nitrite oxidizing organisms at (a) 35°C, (b) 40°C, (c) 45°C, (d) 50°C and (e) 55°C. Black squares represent the NH_4^+-N concentrations; green squares: NO_3^--N concentrations; and, blue diamonds: NO_2^--N concentrations. Green squares may appear light gray in black and white printouts.

Figure 3 - Model simulations of the denitrifying activities over nitrate and nitrite with methanol (MeOH) as external carbon source observed in the batch activity tests executed at (a) 35°C, (b) 40°C, (c) 45°C, (d) 50°C and (e) 55°C. Violet circles represent the MeOH concentrations; black squares: NO_3^--N concentrations; and, blue diamonds: NO_2^--N concentrations. Violet circles may appear light gray in black and white printouts.

Figure 4 - Bacterial population distribution observed in the mixed liquor sample from the oil-refinery by applying Fluorescence *in situ* Hybridization (FISH) (bar indicates 20 μm): (a) *Ammonia oxidizer halotolerant* and *obligatehalopjhilic* (NEU653): green, and EUBacteria: blue; (b) *Nitrosomonas Oligotropha* lineage (NOLI 191): green, and EUBacteria: blue.

Table 1. Fluorescence *in situ* Hybridization (FISH) oligonucleotide probes used in this study for the microbial identification of ammonia- and nitrite-oxidizing organisms.

Probe	Target organism		Reference
EUB mix	All bacteria	*Eubacteria, Planctomyces branch (suplemment for EUB338), Verrucomicrobia (suplemment for EUB338)*	Amman *et al*. (1990), Daims *et al*. (1999)
Nm 75	Ammonia oxidizers	*Nitrosomonas genus*	Hiorns *et al*. (1996)
Nse1472		*Nitrosomonas europaea, Nitrosomonas eutropha, Nitrosomonas halophila*	Juretschko *et al*. (1998)
NOLI 191		*Nitrosomonas oligotropha lineage*	Gieseke *et al.* (2001)
Nso 1225		*Ammonia oxidizing Beta- proteobacteria*	Mobarry *et al*. (1996)
Nso 190		*Ammonia oxidizing Beta- proteobacteria*	Mobarry *et al*. (1996)
Neu 653		*Nitrosomonads (halotolerant and obligatehalophilic) (applied with NEU653comp)*	Wagner *et al*. (1995)
NmV		*Nitrosoccus mobilis*	Juretschko *et al*. (1998)
Nsv 443		*Nitrosospira, Nitrosolobus, Nitrosovibrio*	Mobarry *et al*. (1996)
Ntspa 712	Nitrite oxidizers	*Phylum Nitrospira (Nitrospira-like organisms) (applied with comp Ntspa 712)*	Daims *et al*. (2000)
NIT 1035		*Nitrobacter (applied with NIT1035comp)*	Wagner *et al*. (1996)
Ntspa 662		*Phylum Nitrospira (Nitrospira-like organisms) (applied with comp* Ntspa 662*)*	Daims *et al*. (2000)
Ntspa 712		*Phylum Nitrospira (applied with comp* Ntspa 712*)*	Daims *et al*. (2000)

Table 2 - A comparison of different Arrhenius temperature coefficients for nitrification and denitrification observed in literature and found and applied in this study.

Culture	Temperature range (°C)	Temperature coefficient	Carbon source	Reference
Nitrification				
Mixed	10-25	1.12	N/A	Henze et al. (1995)
Mixed	10-25	1.05	N/A	Smith et al. (2004)
Mixed	35-50	1.03	N/A	This study
Mixed	35-50	1.02	N/A	This study
Denitrification				
Pure	6-30	1.13	Methanol	(Timmermans and van Haute, 1983)
Mixed	7-15	1.06	Influent wastewater	(Oleszkiewicz and Berquist, 1998)
	2-7	1.30		
Pure	15-25	1.11	Methanol	(Christensson and Welander, 1994)
		1.12	Ethanol	
Mixed	10-25	1.07	-	Henze et al. (1995)
Mixed	7-17	1.06	Methanol	Nyberg *et al.* (1992)
		1.06	Ethanol	
Mixed	6-10	1.37	Methanol	(Barlindhaug and Odegaard, 1996)
Mixed	10-25	1.10	Methanol	(Carrera *et al.*, 2003)
Mixed	35-55	1.04	Methanol	This study[1]
Mixed	35-55	1.07	Methanol	This study[2]

[1.] Denitrification over nitrite

[2.] Denitrification over nitrate.

Figure 1
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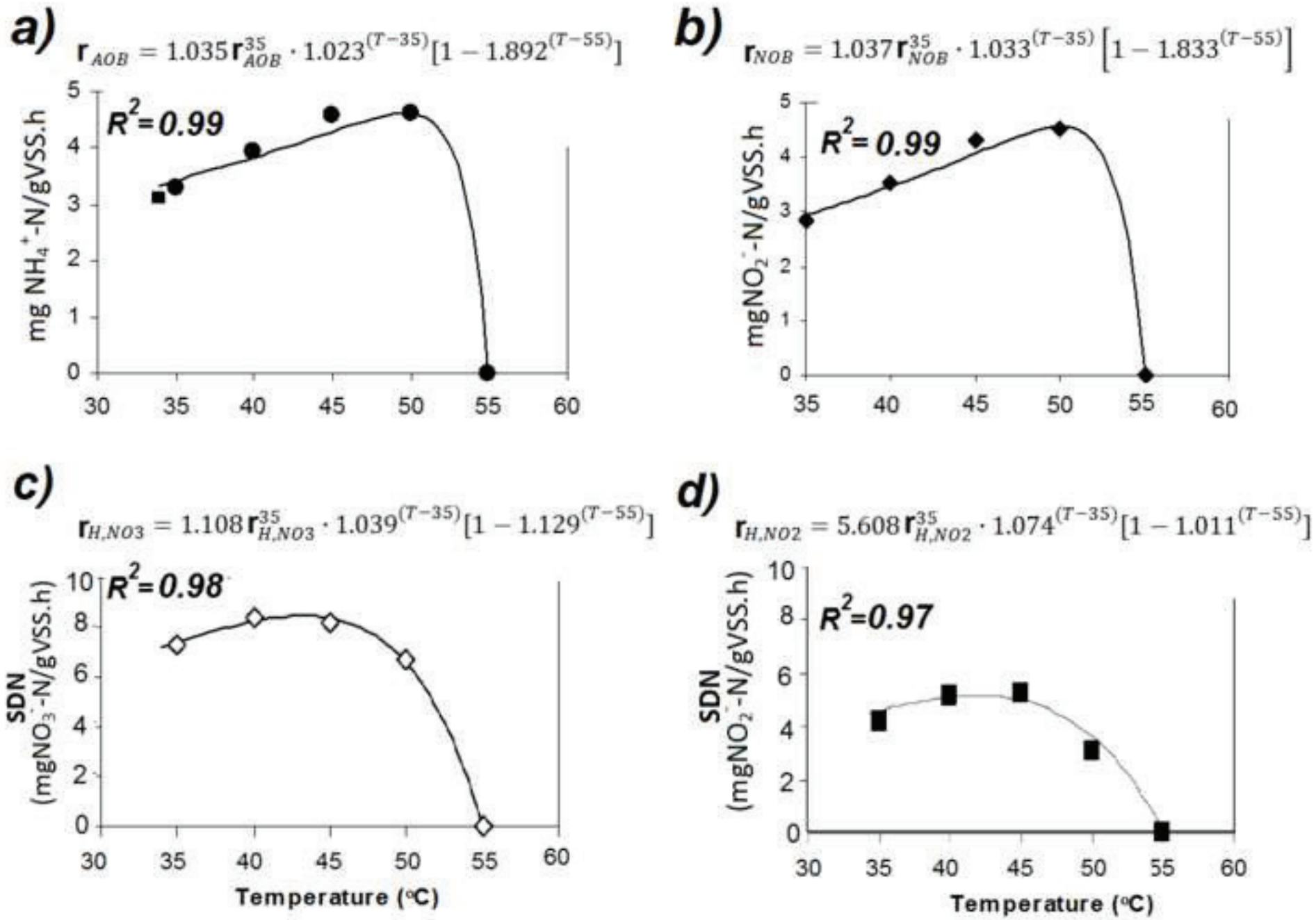

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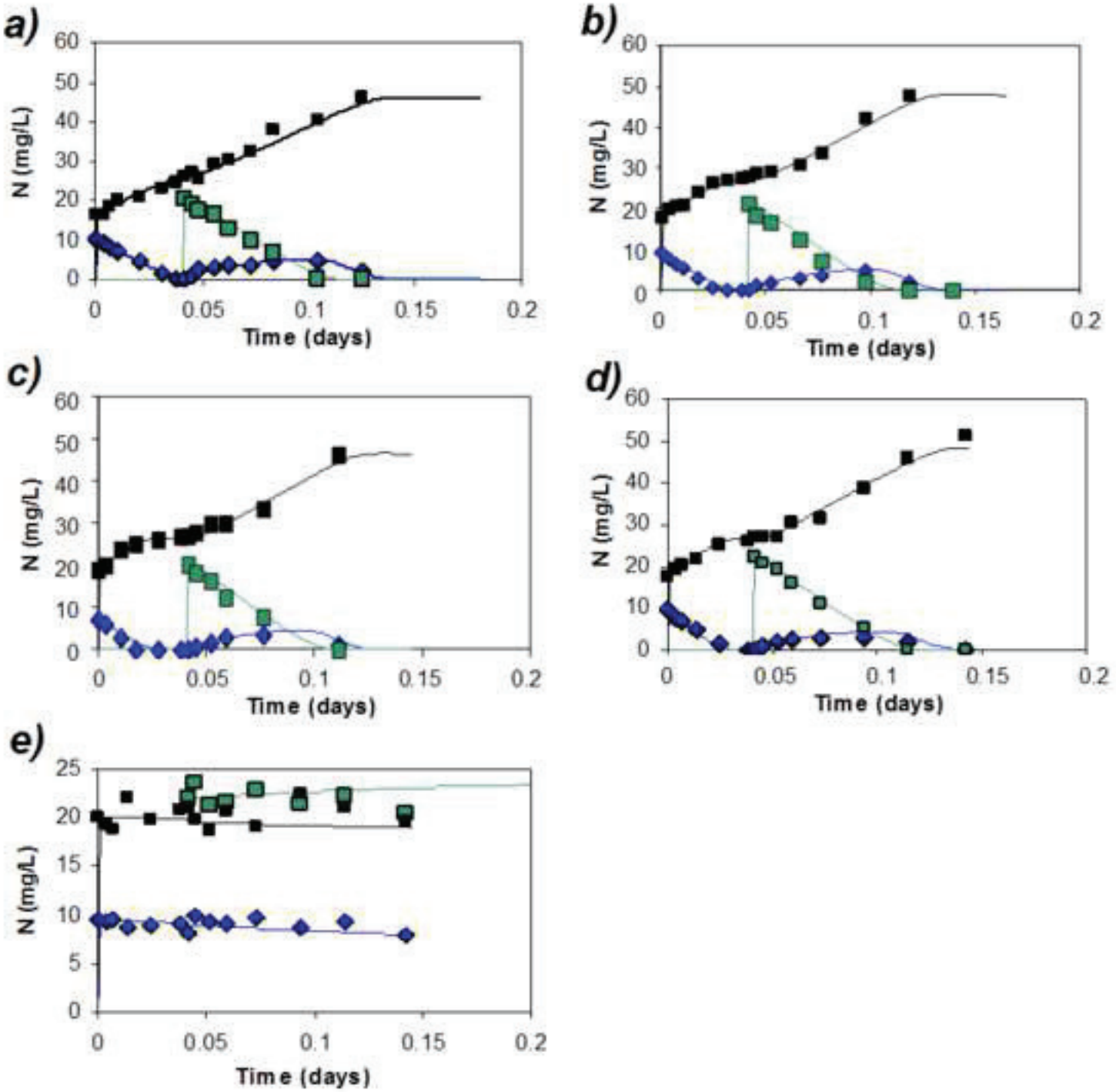

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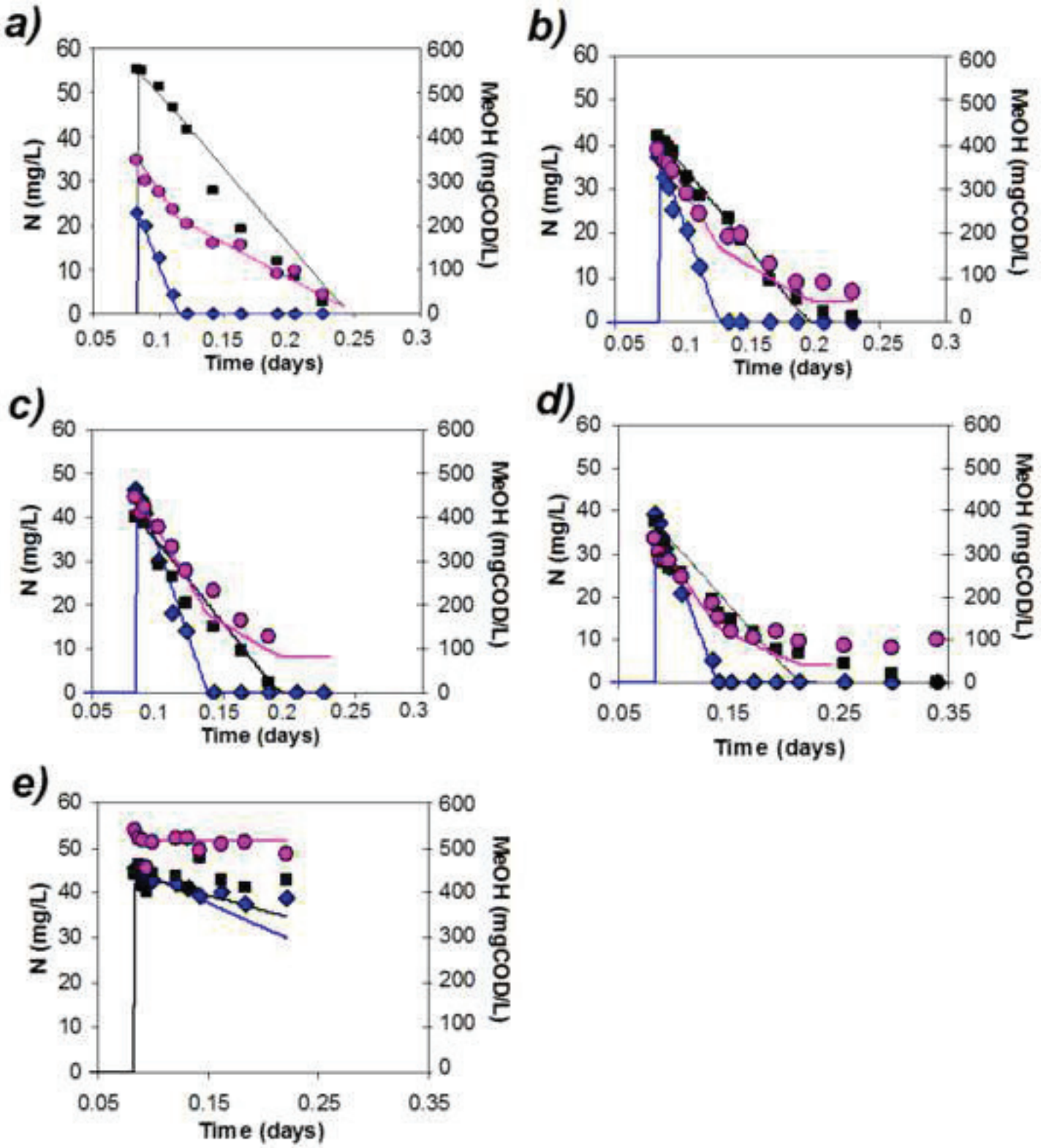

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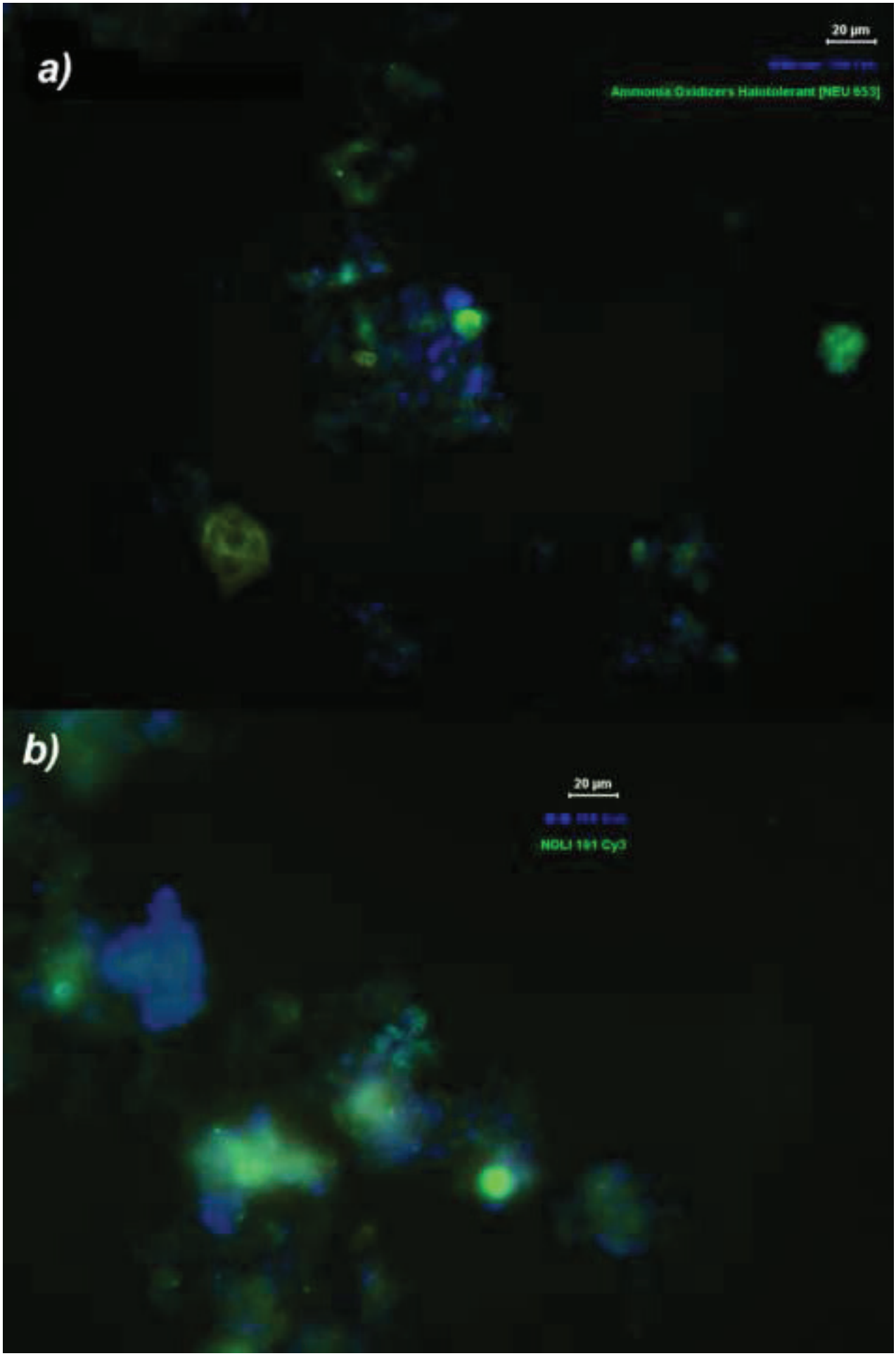

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