

Distillery fusel oil as an alternative carbon source for denitrification – from laboratory experiments to full-scale applications

J. Makinia, K. Czerwionka, M. Kaszubowska and J. Majtacz

ABSTRACT

Industrial waste products may be a viable alternative to commercial external carbon sources. In this study, the properties of fusel oil (distillery waste product) were investigated in terms of temperature dependency of denitrification and acclimation period. Furthermore, results obtained during three different full-scale trials were used to verify similarities with and differences from the results obtained under laboratory-scale conditions. Batch experiments with non-acclimated biomass revealed a very strong temperature dependency of the denitrification process ($\theta = 1.15$, $R^2 = 0.92$) in the range of examined process temperatures (13–22 °C). Fusel oil had minor (or no) effects on the behaviour of NO₂-N and PO₄-P. Significantly lower nitrate utilization rates were observed during acclimation to fusel oil in the full-scale bioreactors compared to a bench-scale reactor. This may primarily be attributed to lower doses of fusel oil, lower process temperatures and more complicated process configurations (resulting in non-optimal use of fusel oil for denitrification). Results obtained from both laboratory-scale experiments and full-scale trials suggested that an acclimation period of a few weeks would be required to reach the maximum denitrification capability of process biomass, even though positive effects of dosing can be observed almost immediately.

Key words | activated sludge, denitrification, external carbon source, fusel oil, nutrient removal

J. Makinia (corresponding author)

K. Czerwionka

M. Kaszubowska

J. Majtacz

Gdansk University of Technology,
Faculty of Civil and Environmental Engineering,
ul. Narutowicza 11/12,
80-233 Gdansk,
Poland
E-mail: jmakinia@pg.gda.pl

INTRODUCTION

Denitrification can be enhanced (in terms of the rate and efficiency) within the existing capacities of biological nutrient removal (BNR) wastewater treatment plants (WWTPs) by adding external carbon sources to anoxic compartments. The application of commercially available compounds (e.g. methanol) is effective, but expensive. In recent years, a shortcut in the nitrogen conversion pathway, i.e. nitrification–denitrification via NO₂-N instead of NO₃-N, has been promoted as an alternative solution in order to reduce the oxygen demand for nitrification and organic carbon demand for denitrification. Fux & Siegrist (2004) noted, however, that significant N₂O production can occur at elevated NO₂-N concentrations in the reactor. Therefore, full denitrification via NO₃-N would be sustainable and economically feasible if a cheap and effective carbon source could be found. Various industrial/agricultural by-products or waste materials are being sought as ‘alternative’ external carbon sources, and their characteristics and applications were

recently summarized by Gu & Onnis-Hayden (2010). These sources comprised raw industrial/agricultural by-products, such as corn syrup, molasses and brewery waste, and other process wastes, such as glycerol from biodiesel production. Also the distillery waste products, including fusel oil, syrup and reject water, were shown to be a viable alternative to commercial external carbon sources, such as ethanol, in both mainstream and sidestream treatment systems performing full nitrification–denitrification (Makinia *et al.* 2009, 2011; Czerwionka *et al.* 2012). Due to advantageous composition resulting from much higher chemical oxygen demand (COD) concentrations and COD/N ratios, fusel oil would be a preferred carbon source for practical handling in full-scale WWTPs. In the literature, there have been other studies (Monteith *et al.* 1980; Klapwijk *et al.* 1981; Zala *et al.* 1999, 2004) reporting successful use of fusel oil for enhancing denitrification. The nitrate utilization rates (NURs) were exceeding 10 g N/(kg VSS · h) in those studies.

A typical fusel oil production during fermentation amounts to 0.2–0.7% of pure ethanol on weight basis. The actual amount and composition of fusel oil produced depends on the raw material used (Alvarez & Hunt 2002). The conventional characteristics of fusel oil, presented by Makinia *et al.* (2011), revealed that it may potentially be a very favourable carbon source for denitrification due to both a high content of soluble, readily biodegradable organic compounds (COD concentrations comparable to ethanol) and a high value of the COD/total nitrogen (TN) ratio (approximately 1,800).

This paper presents further results of a 3-year research and development project concerning the use of fusel oil as a carbon source at large municipal WWTPs. The project was carried out in cooperation with the Water Environment Research Foundation Nutrient Removal Challenge Program. The previous laboratory-scale investigations with non-acclimated process biomass were related to: (1) the immediate effects of dosing fusel oil on the denitrification capability of non-acclimated process biomass and interactions with enhanced biological P removal (Makinia *et al.* 2009); and (2) the potential of using fusel oil in treatment of sludge digester liquors (Makinia *et al.* 2011). This study primarily focuses on the temperature dependency of denitrification with fusel oil and acclimation period required to reach the maximum denitrification capability. With regard to the latter issue, results obtained during three different full-scale trials were compared with the results obtained under laboratory-scale conditions.

METHODOLOGY

Laboratory experiments with non-acclimated process biomass

Two types of batch experiments were carried out with non-acclimated process biomass from a large (600,000 population equivalent, PE) BNR WWTP located in the city of Gdansk (northern Poland). In the one-phase experiments under anoxic conditions (the conventional NUR measurements), nitrate (KNO_3) and fusel oil were injected at the beginning of the test which was run for 6–7 h. In the two-phase anaerobic/anoxic experiments, the process biomass and settled wastewater were mixed and kept under anaerobic conditions for 2.5 h before injecting KNO_3 and fusel oil. The duration of the follow-up anoxic phase was 4 h. Details about the laboratory apparatus and sampling methodology were previously presented by Makinia *et al.* (2011).

Acclimation of process biomass to fusel oil under laboratory conditions

The acclimation of process biomass to fusel oil was investigated in a bench-scale Johannesburg (JHB) system ($V = 30 \text{ dm}^3$) fed with the settled wastewater from the Gdansk WWTP (Figure 1). The system was operated for over 100 days between March and June. The process temperature was gradually increased from 13 to 20 °C. Fusel oil was continuously added to the anoxic compartment at the rate of 1.5 cm^3/d . The mixed liquor recirculation was first set to 500% of the influent flow rate and increased to 600% in the final stage of the experiment (on day 93). The solids retention time (SRT) was kept at a constant level of 20 d. As a consequence, the mixed liquor suspended solids (MLSS) concentrations were varied in the range of 3.2–3.6 kg/m^3 . The set point for dissolved oxygen concentration in the aerobic compartment was set to 2 $\text{g O}_2/\text{m}^3$. The concentrations of principal parameters, including TN, $\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$, $\text{NO}_2\text{-N}$, total phosphorus (TP), $\text{PO}_4\text{-P}$ and COD, were determined on a regular basis, i.e. twice a week, in both influent and effluent grab samples. Some parameters ($\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$ and $\text{PO}_4\text{-P}$) were determined once a week inside the reactor in the anaerobic, anoxic and aerobic compartments. Moreover, eight conventional NUR measurements were carried out on a regular basis, i.e. every 1–3 weeks, with the process biomass during the operation of the system.

Full-scale experiments with dosing fusel oil to the mainstream reactors

Full-scale experiments with fusel oil were carried out at three large BNR WWTPs (JHB-type process configurations) located in the cities of Gdynia, Poznan and Jastrzebie-Zdroj. The schematic layouts of the bioreactors and the dosing points are shown in Figure 2, whereas routine operating data from the plants are listed in Table 1.

In the Gdynia and Poznan WWTPs, the average dosage rate to a selected test line was approximately 0.4 m^3/d , and

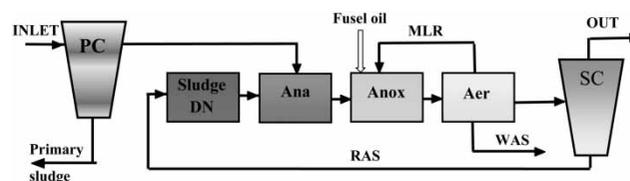


Figure 1 | Layout of the bench-scale JHB system for investigating the acclimation of process biomass to fusel oil (PC – primary clarifier, Sludge DN – sludge denitrification, Ana – anaerobic, Anox – anoxic, Aer – aerobic, SC – secondary clarifier, RAS – returned activated sludge, WAS – wasted activated sludge).

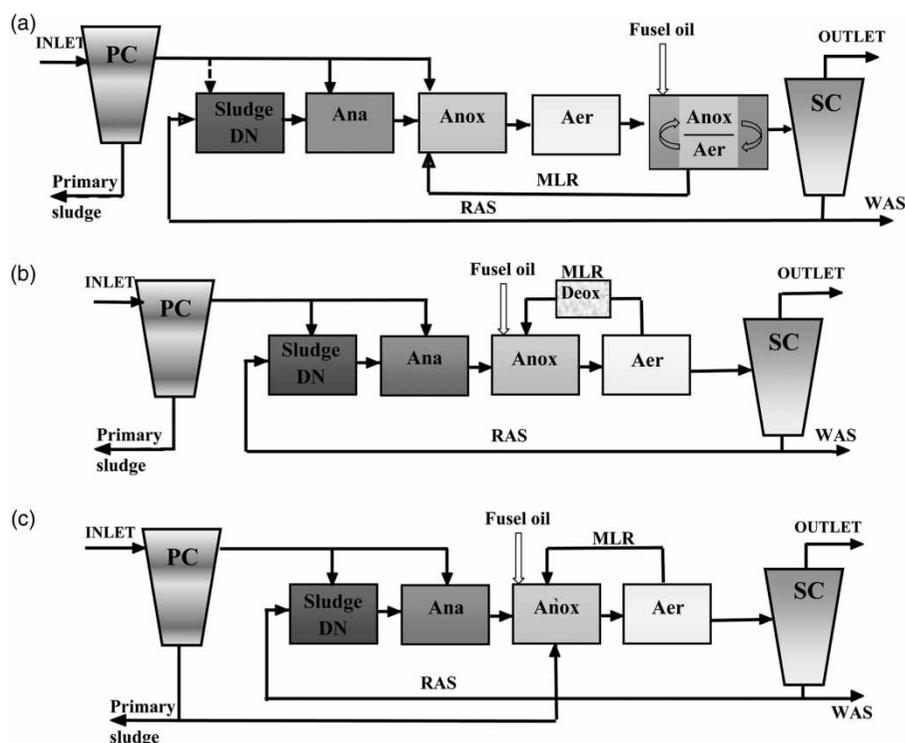


Figure 2 | Layouts of the full-scale JHB systems for investigating the acclimation of biomass to fusel oil: (a) Gdynia WWTP, (b) Poznan WWTP, (c) Jastrzebie-Zdroj WWTP (locations of the dosing points are shown, Deox – deoxic, and the other abbreviations are described in Figure 1).

Table 1 | Routine operating data from the three studied plants (average values from the study periods)

Parameter	Unit	Gdynia	Poznan	Jastrzebie Zdroj
Size	PE	440,000	210,000	130,000
Average flowrate	m ³ /d	56,000	35,000	11,800
SRT	d	22–40	20–36	21–31
<i>Influent</i>				
COD _{Cr}	g COD/m ³	1,125	851	1,033
N _{tot}	g N/m ³	87	72	100
P _{tot}	g P/m ³	8.9	10.1	14.2
<i>Effluent</i>				
COD _{Cr}	g COD/m ³	25.4	26.2	38.7
NO ₃ -N	g N/m ³	5.6	5.4	6.2
NH ₄ -N	g N/m ³	0.8	0.3	1.65
N _{tot}	g N/m ³	7.9	7.8	9.2
P _{tot}	g P/m ³	0.7	0.5	0.4

NO₃-N concentrations were continuously monitored in the test line and reference line (which was one of the lines without dosing fusel oil). The difference between the plants is that the returned activated sludge (RAS) is mixed for all the lines

(Gdynia) or kept separated for each line (Poznan). The Jastrzebie-Zdroj WWTP has only one treatment line so it was not possible to investigate performance of the test and reference lines. Instead of this, fusel oil was added at variable (increased) rates to compensate for more efficient precipitation of organic particulates in the primary clarifier.

In addition to the *in situ* measurements in the full-scale bioreactors, conventional NUR measurements were carried out on a regular basis, i.e. once a week, under laboratory conditions including:

- batch reactor 1: process biomass from the test line, with dosing fusel oil
- batch reactor 2: process biomass from the test line, without dosing fusel oil
- batch reactor 3: process biomass from the reference line, with dosing fusel oil (only the Poznan WWTP).

Analytical methods

The concentrations of inorganic N forms (NH₄-N, NO₃-N and NO₂-N), P forms (TP and PO₄-P) and COD were determined spectrophotometrically with a XION 500 using Dr. Lange cuvette tests (Dr. Lange GmbH, Berlin, Germany).

The TN concentrations were determined using a TOC analyser TOC-V_{CSH} coupled with a TN module TNM-1 (SHIMADZU Corporation, Kyoto, Japan). The analytical procedures, which were adopted by Dr. Lange and SHIMADZU, followed *Standard Methods* (APHA 1992). The concentrations in samples of mixed liquor were determined after filtering the samples under vacuum pressure through a 1.2 μm pore size nitrocellulose filter (Millipore, Billerica, MA, USA). Total suspended solids (TSS) and volatile suspended solids (VSS) were measured by the gravimetric method according to the Polish Standards PN-72/C-04559 (samples from the laboratory experiments and sampling points inside reactors).

Ten samples of fusel oil obtained from seven distilleries were analysed for the presence of 29 specific compounds using gas chromatography with a flame ionization detector Autosystem XL (Perkin Elmer Inc., USA). Helium was used as a mobile phase with three types of column inlet for determination of the contribution of different compounds: HP-INNOWAX at 260 °C (acetic acid); HP-5 at 325 °C (decyl acetate, octyl acetate and dodecyl acetate); and DB-624 at 300 °C (the other compounds).

RESULTS AND DISCUSSION

Fusel oil characteristics

Results of the detailed characteristics of fusel oil with the gas chromatography and theoretical oxygen demands (ThOD) of the most important components are shown in Figure 3. It was found that 2-methyl-1-butanol was a dominating component (almost 40% by weight). The other important components included: 2-methyl-1-propanol, 3-methyl-1-butanol and ethanol. Almost 30% of the overall content was not recognized. It has been reported in the literature (Alvarez & Hunt 2002) that although over 50 different

compounds have been identified in fusel oil, the major components were fermentation amyl alcohols (such as 2-methyl-1-butanol and 3-methyl-1-butanol), other alcohols, acids, esters and aldehydes.

Laboratory experiments with non-acclimated process biomass

Comprehensive laboratory experiments with fusel oil were conducted at the Gdansk WWTP. In the conventional denitrification tests with the support of fusel oil, the measured NURs ranged from 0.6 to 1.3 g N/(kg VSS·h) at the $\Delta\text{COD}:\Delta\text{N}$ ratio of 5.9 ± 0.83 g COD/g N. These results revealed a very strong temperature dependency of the denitrification process ($\theta = 1.15$, $R^2 = 0.92$) in the range of examined process temperatures (13–18 °C). The earlier results from similar measurements (Makinia et al. 2009) also confirmed the estimated temperature dependency for higher temperatures (21–22 °C) (Figure 4). It should be noted that the θ value of 1.15 seems very high in comparison with the default values of 1.07 and 1.03 for, respectively, ‘ordinary’ heterotrophs and polyphosphate-accumulating organisms in the activated sludge models (Henze et al. 2000). However, comparable θ values, i.e. 1.12, 1.15 and 1.2 for, respectively, methanol, raw wastewater and endogenous sources, have been reported in the literature (Bever et al. 1995).

During all the experiments, fusel oil had minor or no effects on the behaviour of $\text{NO}_2\text{-N}$ and $\text{PO}_4\text{-P}$ as their concentrations did not exceed 0.1 g N/m³ and 0.4 g P/m³, respectively. For comparison, in similar experiments with the settled wastewater, the initial release of $\text{PO}_4\text{-P}$ was observed until the readily biodegradable substrate was present in the solution. $\text{NO}_2\text{-N}$ accumulations up to 5 g N/m³ occurred in the first phase of the test and $\text{NO}_2\text{-N}$ was subsequently utilized in the second phase. Such a behaviour of $\text{NO}_2\text{-N}$ may suggest a different denitrification pathway

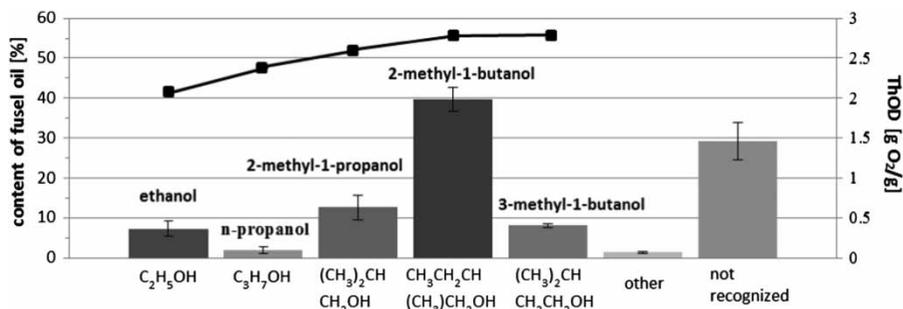


Figure 3 | Average composition of 10 samples of fusel oil from seven different distilleries (columns) and theoretical oxygen demands (ThOD) of the most important components (points).

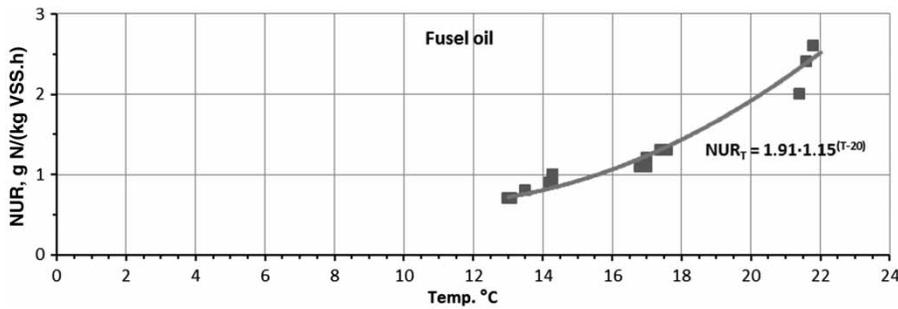


Figure 4 | Temperature dependency of the NURs measured in the experiments with fusel oil as an external carbon source.

resulting from specific substrates present in real wastewater, which are not components of fusel oil.

When adding fusel oil at the beginning of the anoxic phase was preceded by an anaerobic phase (biomass mixed with the settled wastewater), the range of observed NURs, i.e. 1.4–3.5 g N/(kg VSS · h), was higher compared to the reference tests without the addition of fusel oil (1.3–2.5 g N/(kg VSS · h)) in the same range of temperatures as above (13–18 °C). The denitrification process was still strongly dependent on temperature but the correlation was poorer ($\theta = 1.09$, $R^2 = 0.39$). No explicit effect of fusel oil on the $\text{PO}_4\text{-P}$ behaviour in the anoxic phase was observed. The anoxic P uptake rates (PURs) in the experiments with and without the addition of fusel oil appeared to vary within similar ranges, i.e. 2.1–3.9 g P/(kg VSS · h) vs. 2.2–3.6 g P/(kg VSS · h).

Acclimation of biomass to fusel oil under laboratory conditions

The behaviour of the effluent $\text{NO}_3\text{-N}$ concentrations during an operation of over 3 months is presented in Figure 5(a); after the initial stabilization, these remained within a relatively narrow range (7–10 g N/m³). The decreasing $\text{NO}_3\text{-N}$ concentrations corresponded well to the increasing NURs, which can be described best by a power function in terms of time (Figure 5(b)). The observed NURs increased from the initial value of less than 2 g N/(kg VSS · h) and reached the maximum of over 6 g N/(kg VSS · h) on day 50 and then stabilized. This increase could be attributed to both acclimation to fusel oil, observed already on day 1, and increasing process temperature. Significantly lower NURs, up to 2 g N/(kg VSS · h), were obtained in the parallel

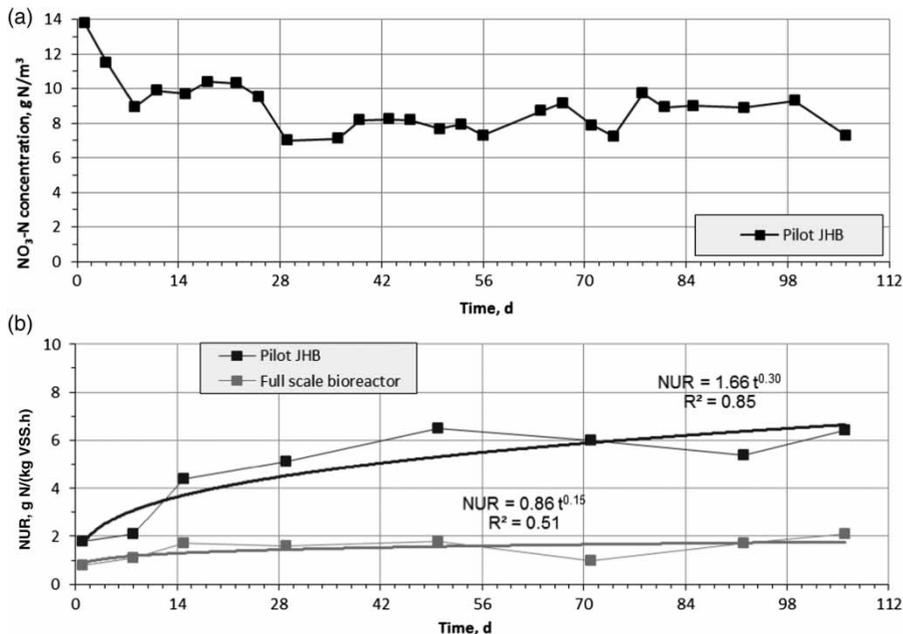


Figure 5 | Results of the acclimation of biomass to fusel oil in the bench-scale JHB system: (a) behaviour of the effluent $\text{NO}_3\text{-N}$ concentrations (grab samples); (b) NURs determined for the mixed liquor taken from the pilot JHB system and full-scale bioreactor.

experiments with the non-acclimated biomass from the full-scale bioreactor. In that case, the effect of the increasing temperature was also observed.

For comparison, a significantly higher increase of NURs was observed in a laboratory-scale sequencing batch reactor (SBR) treating fusel oil and sludge digester liquor in the conventional nitrification–denitrification process. In that case, the rates were continuously increasing and reached the maximum of approximately 15 g N/(kg VSS · h) after a 3-week acclimation period (Makinia *et al.* 2011). Such high NURs could not be obtained in the mainstream bioreactor for three major reasons: (1) more complex carbon sources present in the settled wastewater; (2) lower influent nitrogen loads; and (3) lower process temperatures (13–20 vs. 24 °C in the SBR).

Full-scale experiments with dosing fusel oil to the mainstream bioreactors

The behaviour of NO₃-N concentrations during the full-scale experiments with fusel oil is presented in Figure 6. At the Gdynia WWTP, the average NO₃-N concentration in the test line was lower by approximately 1 g N/m³ compared to the reference line (Figure 6(a)). The observed NURs in the test line (with addition of fusel oil during the test) stayed at a constant level of 1.5 g N/(kg VSS · h) and were approximately three to four times higher compared to the rates without the addition of fusel oil during the test (Table 2). At the Poznan WWTP, the effect of dosing fusel oil to the test line was also apparent (Figure 6(b)). Based on the observed NURs, it was estimated that a 3-week acclimation period

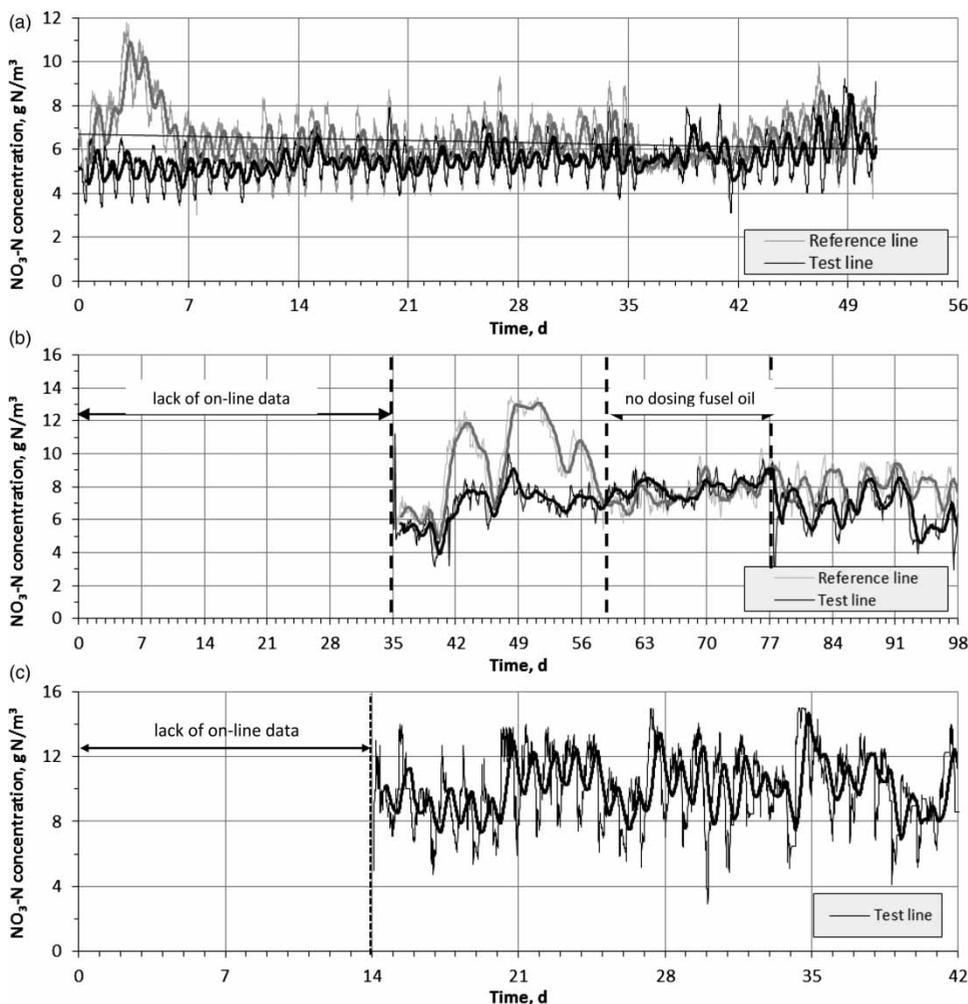


Figure 6 | NO₃-N concentrations during the full-scale experiments with fusel oil at the three studied WWTPs: (a) Gdynia, (b) Poznan, (c) Jastrzebie-Zdroj (bold lines – moving averages with intervals of 80 (Gdynia) and 20 (Poznan and Jastrzebie-Zdroj)).

Table 2 | Summary of the NUR measurements in batch experiments with or without adding fusel oil at the three studied WWTPs

WWTP	Biomass from the test line NUR, g N/(kg VSS · h)		Biomass from the reference line NUR, g N/(kg VSS · h) With dosing fusel oil
	With dosing fusel oil	Without dosing fusel oil	
Gdynia	1.2–1.6	0.3–0.5	Not determined
Poznan	1.7–2.7	0.7–1.0	1.3–1.5
Jastrzebie Zdroj	1.9–3.4	0.3–0.9	Not determined

was required to reach the maximum denitrification capacity of process biomass. A similar acclimation period was also found at the Jastrzebie–Zdroj WWTP (Figure 6(c)). For these two plants, as in the bench-scale JHB system, the acclimation was described by power functions:

$$\text{NUR} = 1.70t^{0.08} (R^2 = 0.74) \text{ Poznan WWTP} \quad (1)$$

$$\text{NUR} = 1.91t^{0.16} (R^2 = 0.93) \text{ Jastrzebie–Zdroj WWTP} \quad (2)$$

It should be emphasized that the COD load originating from the fusel oil compared to the influent COD load was relatively stable, i.e. 12–15.3%, at the two plants (Gdynia and Poznan) and similar to the bench-scale JHB system (13.7%). At the Jastrzebie–Zdroj WWTP, this ratio increased from 13 to 30%. The aim of this specific operation was to replace the particulate COD removed more efficiently by coagulation–flocculation in the primary clarifier.

Table 2 presents the NURs observed in the three studied plants and from these results it is apparent that the effect of dosing fusel oil was different in each case. This may suggest differences in both the level of acclimation of the process biomass to fusel oil and the significance of the composition of raw wastewater. Furthermore, the observed NURs in the studied full-scale bioreactors were significantly lower in comparison with the rates observed in the bench-scale JHB system. This may be attributed to lower doses of fusel oil, lower process temperatures and more complicated process configurations, resulting in non-optimal use of fusel oil for denitrification.

CONCLUSIONS

Based on the results of this study, the following conclusions may be derived:

- It has been confirmed in the full-scale studies that fusel oil, which is a distillery by-product, may be a viable alternative for commercial compounds, such as ethanol, when seeking an external carbon source for denitrification in activated sludge systems. However, the NURs with fusel oil appear to be strongly influenced by temperature ($\theta = 1.15$).
- Both laboratory-scale experiments and full-scale trials suggested that an acclimation period of a few weeks would be required to reach the maximum denitrification capability of process biomass, even though positive effects of dosing can be observed almost immediately.
- Lower NURs in the mainstream reactors may be expected in comparison with sidestream systems (using the conventional nitrification–denitrification processes) which may primarily result from more complex carbon sources present in the settled wastewater and lower influent nitrogen loads.

ACKNOWLEDGEMENT

This study has been financially supported by the European Regional Development Fund within the framework of the Innovative Economy Operational Programme 2007–2013 under the project no. UDA-POIG.01.03.01-22-140/09-04.

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First received 1 November 2013; accepted in revised form 14 January 2014. Available online 25 January 2014