

Estimating the impact of inhibitory substances on activated sludge denitrification process

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Abstract

Industrial wastewater frequently contains substances which inhibit activated sludge treatment processes. Inhibitory characteristics of different substances are usually evaluated based on testing the impact of respective substance on activated sludge nitrification or oxygen uptake rates. However, denitrification is always before aerobic processes in conventional activated sludge treatment plants and thereby more exposed to inhibitory compounds. There is no easily applicable and validated method available for determination of denitrification process efficiency and inhibition. In this study, a method for evaluation of inhibition on the activated sludge denitrification process was developed and validated using 3,5-dichlorophenol (3,5-DCP) as a model inhibitory compound and additionally controlled with real wastewater produced in the shale oil industry. Average IC_{50} value ($5.5 \pm 2.2 \text{ mg L}^{-1}$) for 3,5-DCP showed that denitrifiers were less sensitive than nitrifiers ($IC_{50} = 2.9 \pm 0.7 \text{ mg L}^{-1}$) and more sensitive than aerobic heterotrophs ($IC_{50} = 7.2 \pm 2.4 \text{ mg L}^{-1}$). Methodological aspects like accumulation of nitrite nitrogen, acclimatization of biomass and technical issues were discussed. Achieved validation characteristics were similar with ISO Standards estimating activated sludge nitrification and oxygen uptake rates, which proves the reliability of the method: standard deviation, 95.4% confidence level, relative standard deviation were calculated to be 2.2 mg L^{-1} , $1.2 \dots 9.8 \text{ mg L}^{-1}$ and 39.2%, respectively.

Key words: activated sludge denitrification, hazardous substances, industrial wastewater, inhibition, validation of method

INTRODUCTION

Biological nitrogen removal from wastewater is conventionally performed via nitrification and denitrification processes. During nitrification, ammonium is biologically oxidized to nitrite (nitritation) and then to nitrate (nitrataion) with O_2 as terminal electron acceptor. These processes are performed by autotrophic bacteria – ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB), respectively. Growth rate of nitrifying microorganisms is low, which makes the nitrification process sensitive to unsuitable conditions (e.g. the presence of different toxic substances in the process) (Philips *et al.* 2002; Haandel & Lubbe 2012).

In the denitrification process, nitrate is reduced stepwise to nitrite (NO_2), nitric oxide (NO), nitrous oxide (N_2O) and molecular nitrogen (N_2) (Equation (1)). Each oxidized nitrogen form is catalyzed by a specific enzyme (NO_3 , NO_2 , NO or N_2O reductase) and attends as an electron acceptor for the denitrifying bacteria respiration, coupled with biodegradable organic compound as electron donor for energy generation. Organisms containing at least two or three of these specific enzymes and producing nitrous oxide or dinitrogen gas will be referred to as ‘denitrifiers’. Nitric oxide is enclosed in brackets in the description of this reaction mechanism because it is still uncertain whether NO is a true intermediate in the process or a side product (Philips *et al.* 2002; Lin *et al.* 2009;

Chen & Strous 2013).



Contrary to the nitrification process, denitrification is accomplished by a relatively wide range of microorganisms (prokaryotic microorganisms, chemoorgano-heterotrophs, chemo-litho-autotrophs). Growth rate of anoxic denitrifying organisms is at the same level as for aerobic heterotrophic organisms, but much greater than for nitrifiers. Thus, denitrifying organisms should be much more resistant to different unsuitable environmental conditions than nitrifiers (Gray 2004; Stasinakis *et al.* 2008). However, in some cases denitrifiers are more susceptible to the inhibitory effect of pollutants (e.g. copper), when compared with nitrifiers. Furthermore, the anoxic denitrification tank is conventionally located before the aerobic nitrification tank, which makes the denitrification process more vulnerable to inhibitory substances in raw wastewater. Incomplete denitrification could be caused by: (a) imbalances in the electron donor supply; (b) reactive radicals produced in biological processes; (c) low/high pH; (d) presence of oxygen; (e) low/high temperature (Oh & Silverstein 1999; Ochoa-Herrera *et al.* 2009; Sun *et al.* 2016).

It has been shown that wastewater treatment processes could be inhibited by different hazardous substances present in wastewater: heavy metals, cyanides, phenols, nanoparticles, different polar and non-polar organic substances etc. (Çeçen *et al.* 2010; Kim *et al.* 2011; Sibag *et al.* 2015; Su *et al.* 2015; Inglezakis *et al.* 2017a; Hernandez-Martinez *et al.* 2018) or by landfill leachate and wastewaters originating from wood and oil industries (Klauson *et al.* 2014, 2015; Klein *et al.* 2017). In order to avoid reduction in treatment efficiency or to detect the origin of inhibitory wastewater, it is necessary to perform inhibition studies for each biological wastewater treatment process separately. Today, several methods are used for this purpose: ISO 8192 for assessing inhibition of oxygen consumption by activated sludge, ISO 9509 for assessing the inhibition on nitrification of activated sludge microorganisms, ISO 9888 for evaluating the aerobic biodegradability of organic compounds (ISO 8192; ISO 9509; ISO 9888). Nevertheless, there is no reliable and easily applicable method for measuring the impact of wastewater or hazardous compound on activated sludge denitrification process.

Quantification of denitrification substantially varies in different environments and at different scales (Groffman *et al.* 2006). Denitrification potential in aquatic and terrestrial environments has been measured applying different methods: acetylene inhibition method; ^{15}N trace isotopes; direct N_2 quantification; $\text{N}_2:\text{Ar}$ ratio quantification; mass balance approaches; stoichiometric approaches; *in situ* gradients with atmospheric environmental tracers and molecular approaches (e.g. Illumina MiSeq sequencing) (Sun *et al.* 2016). Inhibition of denitrification of activated sludge could be measured by few different techniques. Mostly, nitrogen isotope techniques and acetylene inhibition method are used (Lin *et al.* 2009). Both methods need advanced instrumentation (gas chromatography, mass spectrometry, atomic emission spectrometry) which are not available in many WWTPs and laboratories. Batch tests are described in some papers where impact of hazardous substances on various activated sludge processes are studied (Ochoa-Herrera *et al.* 2009; Inglezakis *et al.* 2017b), but there is lack of information about calculation of results and validation parameters of the method. Therefore, comparing different denitrification inhibition studies is complicated.

The aim of this study was to develop, validate and test an easily applicable and reliable method for evaluation of activated sludge denitrification process. The method gives a possibility to measure the inhibition of wastewater or hazardous substances on the activated sludge denitrification process without advanced equipment.

MATERIALS AND METHODS

Analytical methods

The standard colorimetric method (APHA, 2012) was used in order to determine the 7-day biochemical oxygen demand (BOD₇) and chemical oxygen demand (COD). pH was measured with an E6115 pH meter (Evikon, Estonia) and concentration of dissolved oxygen with Marvet Junior 2000 portable oxygen analyser (Elke Sensor, Estonia). NO₃-N and NO₂-N were determined by spectrophotometric methods according to SFS 5752 and SFS 3029, respectively. Dissolved organic carbon (DOC) was measured with Analytik Jena Multi N/C 3000 TOC analyser. NH₄⁺-N was determined using the HACH-Lange spectrophotometric method with Nessler reagent (ISO 7150-1). The concentration of phenols was measured using a sodium nitrite method with HACH-Lange cuvette tests LCK 345. The results of all the analyses were expressed as the mean with standard deviations of at least 3 parallel replicates.

Set-up of experiment

Activated sludge samples taken from a municipal WWTP (100 000 PE) were used as an inoculum unless stated otherwise. Firstly, activated sludge was washed with tap water at least three times by applying settling period and subsequent decanting. 500 mL activated sludge mixture contained 1 mL phosphate buffer, 1 mL MgSO₄ × 7H₂O, 1 mL CaCl₂ × 7H₂O, 1 mL FeCl₃ × 6H₂O, 50 mL NaHCO₃ solution (2.52 gL⁻¹) and 2 mL acidic microelements, 2 mL basic microelements. Recipes of acidic and basic microelements were taken from Zhang *et al.* (2009).

Experiments were performed in 300 mL biochemical oxygen demand (BOD) bottles. 200 mgL⁻¹ Na-acetate, 30 mgL⁻¹ NO₃-N and 6 different concentrations of toxicant were added to the bottles. Seventh bottle was a blank control and did not contain toxicant. Finally, 50 mL of adapted activated sludge mixture was added into each bottle. Continuous stirring was applied during the experiment. First samples (starting point) were collected and centrifuged immediately after addition of inoculum. Bottles were purged with N₂ for 20–30 minutes and sealed air tightly. Concentration of dissolved oxygen and value of pH were monitored during the process. Following samples were taken after 1 hour and 3 hours under anoxic conditions. Purging with N₂ is required after every sampling to ensure anoxic conditions in test bottles. All samples were taken from fully-mixed samples to ensure that sampling would not change the MLSS value. NH₄⁺-N, NO₃-N, NO₂-N, DOC and pH were analyzed from centrifuged samples. DOC was measured to ensure that inhibition was not resulted from carbon deficiency. Experiments were performed at ambient temperature. The percentage of inhibition of the denitrification is estimated by comparison of the rate with that of a control mixture containing no inhibitor.

It is essential that denitrification process is viable in control, but it is also important that small concentration nitrate is left at the end of the test period to ensure that the substrate was not limiting the process.

The percent of denitrification inhibition was calculated as following:

$$R_d = \frac{(C_{1,N} - C_{3,N})}{MLSS} \times \Delta t \quad (2)$$

$$I_d = \frac{(R_{d,0} - R_{d,x})}{R_{d,0}} \times 100\% \quad (3)$$

R_d – denitrification rate (mgNO_x-N × gMLSS⁻¹ × h⁻¹)

R_{d,0} – denitrification rate in the control vessel (mgNO_x-N × gMLSS⁻¹ × h⁻¹)

R_{d,x} – denitrification rate in the test vessel (mgNO_x-N × gMLSS⁻¹ × h⁻¹)

c_{1,N} – total concentration of nitrate after one hour of test period (mgL⁻¹)

$c_{3,N}$ – total concentration of nitrate after three hours of test period (mgL^{-1})

MLSS – concentration of mixed liquid suspended solids (gL^{-1})

Δt – duration of test (h)

I_d – inhibition of denitrification (%)

Inhibition tests were done in seven parallel replicates during the control of the developed method; in three parallel replicates when the method was used after the control and in three parallel replicates when ISO Standards were applied. OriginPro 8.0 was used for dose-response analysis of data.

Characteristics of toxic compounds

Two different matrixes were used as toxicants to develop and control the method. Firstly, tests were performed with 3,5-dichlorophenol (3,5-DCP) solution (1 gL^{-1}). 0–12.5 ml of 3,5-DCP solution was added to achieve toxicant concentrations 0–50 mgL^{-1} . Chlorophenols and their derivatives are persistent and highly toxic environmental pollutants which are used in the manufacture of several industrial products. 3,5-dichlorophenols are formed via dehalogenation of polychlorophenols (Villemur 2013; Arora & Bae 2014). Additionally, 3,5-DCP has been used as a reference compound in ISO 9509 (toxicity test for assessing the inhibition of nitrification of activated sludge microorganisms), ISO 8192 (test for inhibition of oxygen consumption by activated sludge) and by Milenkovski *et al.* (2010).

Secondly, real wastewater from the shale oil industry was tested. Wastewater samples were taken in September 2016 with its parameters shown in Table 1. The wastewater has a high content of organic compounds (expressed as COD and BOD_7), phenols and ammonium. However, the content of phosphorous was below the limit of detection and it was not required to add supplementary phosphorous because the activated sludge mixture contained a phosphate buffer. The wastewater was highly inhibitory to the activated sludge nitrification rate and oxygen uptake rate. Inhibition of phenolic wastewater to wastewater treatment processes could be caused by several aspects. Some metals that are present in wastewater can catalyze the production of hydroxyl radicals and promote stress through redox cycling activity and damage the cell membrane functions. Additionally, the presence of aromatic compounds could reduce the affinity of the microbial cells for the carbon source and change the structure of the cell (Chen *et al.* 2009).

RESULTS AND DISCUSSION

Effect of 3,5-dichlorophenol on nitrification and oxygen uptake rates

Addition of a toxic concentration of a test material results in a decrease in the wastewater treatment process rate. 3,5-DCP is a test material that has been used for validation of tests for inhibition of

Table 1 | Parameters of the raw wastewater ($n = 3$)

| Parameter | Unit | Value | Standard deviation |
|--|--------------------|--------|--------------------|
| BOD_7 | mg L^{-1} | 6,425 | 378 |
| COD | mg L^{-1} | 11,050 | 340 |
| BOD_7/COD | – | 0.58 | 0.05 |
| Total phenols | mg L^{-1} | 470 | 2 |
| pH | – | 6.0 | 0.1 |
| NH_4^+-N | mg L^{-1} | 390 | 5 |
| $\text{PO}_4^{3-}-\text{P}$ | mg L^{-1} | <0.02 | – |
| Inhibition on nitrification rate, IC_{50} | % | 0.7 | 0.1 |
| Inhibition on oxygen uptake rate, IC_{50} | % | 4.5 | 0.2 |

oxygen consumption by activated sludge (ISO 8192) and toxicity test for assessing the inhibition of nitrification of activated sludge microorganisms (ISO 9509). According to the standards, $9.3 \pm 3.7 \text{ mgL}^{-1}$ of 3,5-DCP inhibits 50% of total respiration (ISO 8192) and $5.6 \pm 3.0 \text{ mgL}^{-1}$ of 3,5-DCP inhibits 50% of nitrification rate (ISO 9509). Based on tests performed during this study, $7.2 \pm 2.4 \text{ mgL}^{-1}$ of 3,5-DCP inhibited 50% of oxygen uptake rate (Figure 1). Average oxygen uptake rate in the blank sample was $7.3 \text{ mgO}_2 \times \text{gMLSS}^{-1} \times \text{h}^{-1}$. $2.9 \pm 0.7 \text{ mgL}^{-1}$ of 3,5-DCP inhibited 50% of nitrification rate with an average nitrification rate of the blank sample $2.5 \text{ mgNO}_3^- \text{-N} \times \text{gMLSS}^{-1} \times \text{h}^{-1}$. Thus, activated sludge used in this study was suitable when compared with the values and uncertainties given in respective standards.

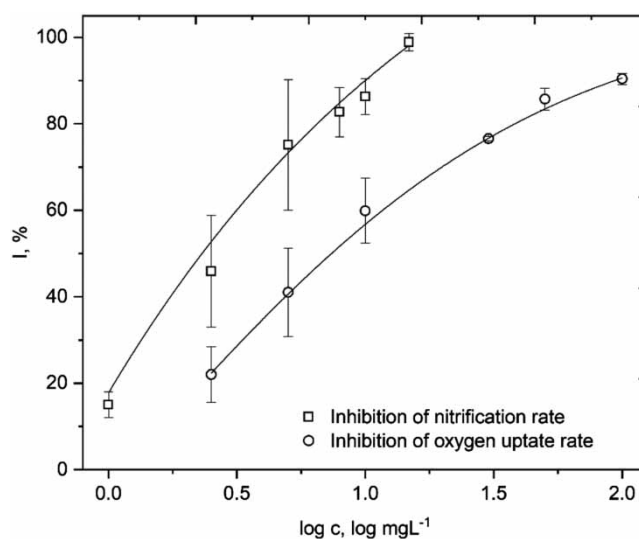


Figure 1 | Inhibition test results for nitrification rate and total respiration by 3,5-dichlorophenol (3,5-DCP). The dark lines show the best dose-response fitting and error bars show the standard deviation of parallel tests ($n = 3$).

Effect of 3,5-dichlorophenol on denitrification rate

An inhibition test was developed in order to measure the impact of any compound or wastewater to activated sludge denitrification process. Method of denitrification inhibition test is described in Section 'Set-up of experiment'. Firstly, developed test was controlled by using 3,5-DCP as a model inhibitory compound. Already 5.5 mgL^{-1} of 3,5-DCP inhibited 50% of denitrification rate. Therefore, 3,5-DCP is inhibitory to denitrification process and is suitable for control of the method. Acetylene gas method used in assessment of the 3,5-DCP inhibition for denitrification in wetland water and sediment has shown EC_{50} value of 34.8 mgL^{-1} (Milenkovski *et al.* 2010).

In denitrification process, nitrate is firstly reduced to nitrite (NO_2^-) and then to gaseous forms (NO , N_2O and N_2). Based on legislations, it is commonly required to measure total nitrogen (TN) in the effluent of WWTP. TN contains organic nitrogen, $\text{NH}_4^+\text{-N}$, $\text{NO}_2^-\text{-N}$ and $\text{NO}_3^-\text{-N}$. Thus, $\text{NO}_2^-\text{-N}$ should also be removed from the wastewater in order to achieve required limits. Therefore, it was tested whether it is necessary to measure $\text{NO}_2^-\text{-N}$ concentrations and add respective values to inhibition calculations. When $\text{NO}_2^-\text{-N}$ concentration is included to calculations, rate of denitrification process is lower and slope of inhibition curve may be higher. In this study, addition of $\text{NO}_2^-\text{-N}$ concentration to the inhibition calculations led to 52% lower value of IC_{50} and 20% lower average denitrification rate in blank sample (Figure 2). Therefore, it is essential to consider values of $\text{NO}_2^-\text{-N}$ in the estimation of denitrification inhibition. Inhibition of NO or N_2O reductase and accumulation of $\text{NO}_2^-\text{-N}$ could be unnoticed if only $\text{NO}_3^-\text{-N}$ values were considered.

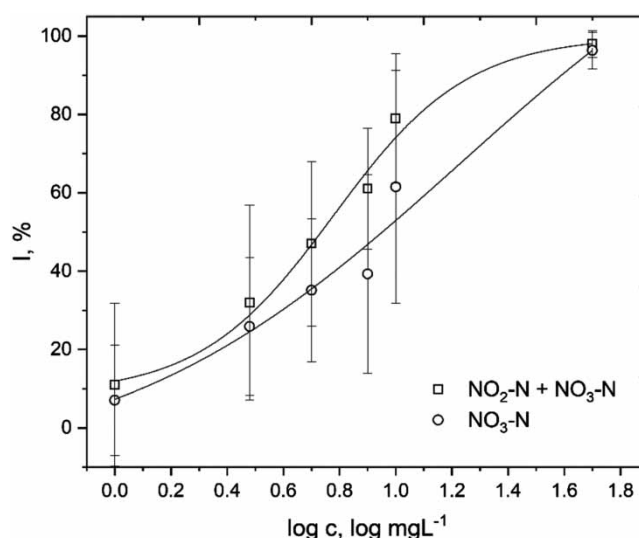


Figure 2 | Test results of denitrification rate by 3,5-dichlorophenol (3,5-DCP) when inhibition calculations include concentration of NO_3^- -N, sum of NO_3^- -N and NO_2^- -N concentrations. The dark lines show the best dose-response fitting and error bars show the standard deviation of parallel tests ($n = 7$).

The second aspect of calculating the potential inhibition of the denitrification rate is the selection of suitable data points. Samples were taken similarly to ISO 9509 – an initial sample after the addition of inoculum and samples after 1 hour and 3 hours of anoxic conditions. However, the denitrification rate was unstable during the first hour of the test period ($1.2 \pm 1.0 \text{ mgNO}_x^- \text{-N} \times \text{gMLSS}^{-1} \times \text{h}^{-1}$), which led to a lower mean denitrification rate and higher standard deviation of denitrification rates calculated from 0–3 hours compared to results from 1–3 hours of the test period (Table 2). The first hour of the test period is an additional adaption phase for denitrifying microorganisms and causes inadequate fluctuation of inhibition test results. Therefore, all inhibition calculations were made from the first to the third hour of the test period to achieve higher accuracy and better repeatability of the method.

Table 2 | Denitrification rates calculated based on NO_x^- -N values at 0–1 hours, 0–3 hours and 1–3 hours of test period ($n = 7$)

| | Unit | 0–1 hours | 0–3 hours | 1–3 hours |
|---------------------------|--|-----------|-----------|-----------|
| Mean denitrification rate | mg NO_x^- -N/gMLSS \times h | 1.2 | 1.6 | 1.9 |
| Standard deviation | mg NO_x^- -N/gMLSS \times h | 1.0 | 1.0 | 0.8 |

Reliability of the method

In order to have comparable results, validation parameters were chosen based on ISO 8192 and ISO 9509 Standards. Validation parameters are shown in Table 3. The achieved average IC_{50} value shows that activated sludge denitrifiers are less sensitive than nitrifiers ($\text{IC}_{50} = 2.9 \pm 0.7$) and more sensitive than heterotrophs ($\text{IC}_{50} = 7.2 \pm 2.4$). ISO 8192 gives an IC_{50} value of $9.3 \pm 3 \text{ mg/L}$ with 95.4% confidence values from 7.1 to 11.3 mg/L. Furthermore, ISO 9509 gives an IC_{50} value of $5.6 \pm 3 \text{ mg/L}$ for 3,5-DCP with 95.4% confidence values from 0.7 to 9.6 mg/L. Thus, a high variation in activated sludge experiments is common and should be considered. It is recommended not to make any final conclusions based on less than three parallel replicates. There are several aspects that could cause higher variability of results in inhibition studies: (1) uncertainties from measurements (2) change of the environment of activated sludge microorganisms (e.g. temperature change in winter months, sludge wash); (3) different activity of activated sludge when tests are performed over long time periods and different seasons. Relatively high uncertainty, which is characteristic of activated sludge

Table 3 | Validation parameters of developed denitrification inhibition method

| Validation characteristic | Unit | Value |
|--------------------------------|--------------------|-------------|
| Number of parallels | – | 7 |
| Average IC ₅₀ value | mg L ⁻¹ | 5.5 |
| Standard deviation | mg L ⁻¹ | 2.2 |
| 95.4% confidence level, k = 2 | mg L ⁻¹ | 1.2 ... 9.8 |
| Relative standard deviation | % | 39.2 |

processes, should be considered when applying the activated sludge denitrification test as well as ISO 8192 and ISO 9509.

Effect of real industrial wastewater on denitrification rate

In addition to the model compound 3,5-DCP, the denitrification inhibition test was performed with real wastewater from the shale oil industry. Characteristics of the wastewater are described in the section ‘Characteristics of toxicants’. Already, $2.6 \pm 0.7\%$ of phenolic wastewater inhibited 50% of the denitrification rate. Denitrifiers were 1.7-fold more sensitive to phenolic wastewater than aerobic heterotrophic microorganisms (Table 1). Biomass adaption should be considered at the time of inoculum selection because waste activated sludge is able to acclimate with inhibitory compounds. Acclimated cultures can tolerate much higher concentrations of an inhibitory compound than non-acclimated systems (Lin *et al.* 2009). The denitrification inhibition test with real industrial wastewater was additionally performed with activated sludge originating from the region of oil shale industries (200,000 PE, proportion of industrial inflow was 70%). Although the sludge should have been acclimated with a higher content of phenols, the studied wastewater showed the same IC₅₀ value as tests performed with activated sludge originating from a conventional municipal WWTP (Figure 3). There could be different reasons behind the result. Firstly, the wastewater from the oil shale industry may have been too toxic for any kind of activated sludge and less harmful solution should be used to show the adaption effect. Secondly, activated sludge samples could have been taken when sludge resistance had decreased because of a disturbance (difference in influent composition, changes in

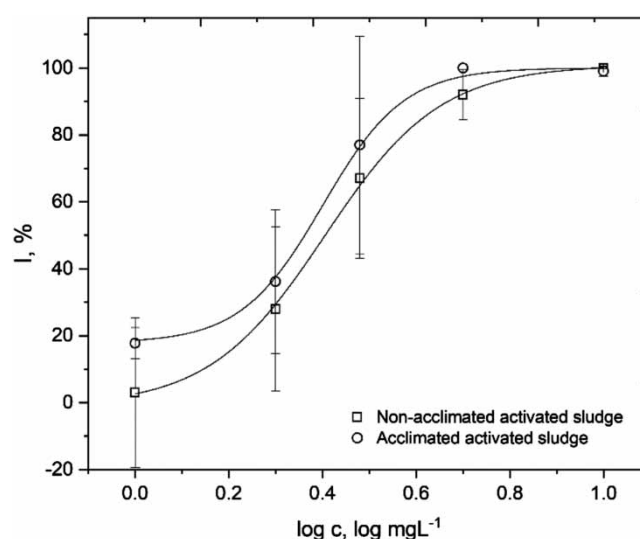


Figure 3 | Test results of denitrification rate with real industrial wastewater from the oil shale industry with inoculum taken from conventional municipal wastewater treatment plant (MWWTP) and from WWTP where activated sludge is acclimated with phenols and error bars show the standard deviation of parallel tests ($n = 3$).

operation of the WWTP). The result would have been different from any other wastewater or sampling period. Since the resistance of activated sludge in a WWTP could change, this method shows the effect of used wastewater or toxic substances on the activated sludge process during the period when the test was taken. Furthermore, although the requirement for NO_2^- -N measurement was studied in the section 'Effect of 3,5-dichlorophenol on denitrification rate', there was no difference in IC_{50} values when calculations considered only the value of NO_3^- -N. It demonstrates that the difference in IC_{50} values depends on the characteristics of the studied model compound or wastewater and it would be incorrect to increase the value of IC_{50} by 52% without real measurements of NO_2^- -N.

CONCLUSIONS

The inhibitory effect of hazardous substances on activated sludge oxygen consumption, nitrification and denitrification processes differs due to different growth rates, resistance, and the process location in WWTPs and should be evaluated separately. Although denitrification is an important but vulnerable process in wastewater treatment, there has been no good method available for evaluating the inhibition of that process. In this study, an easily applicable method for measuring the inhibition of the activated sludge denitrification process was developed and controlled. Validation of the method was performed with model substance 3,5-dichlorophenol (3,5-DCP) and real wastewater from the shale oil industry. This method could be used for evaluation of inhibition caused by industrial wastewater or for measuring the inhibitory characteristics of specific substances or mixtures. The achieved validation parameters are in accordance with values given in ISO 8192 and ISO 9509 standards.

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REFERENCES

- APHA (American Public Health Association) 2012 *Standard methods for the examination of water and wastewater*, 22nd edn. American Water Works Association, Water Environment Federation, Washington, DC, USA.
- Arora, P. K. & Bae, H. 2014 *Bacterial degradation of chlorophenols and their derivatives*. *Microbial Cell Factories* **13**(1), 1–17.
- Çeçen, F., Semerci, N. & Geyik, A. G. 2010 Inhibitory effects of Cu, Zn, Ni and Co on nitrification and relevance of speciation. *Journal of Chemical Technology and Biotechnology* **85**(4), 520–528.
- Chen, J. & Strous, M. 2013 *Denitrification and aerobic respiration, hybrid electron transport chains and co-evolution*. *Biochimica et Biophysica Acta (BBA) - Bioenergetics* **1827**(2), 136–144.
- Chen, H. L., Yao, J., Wang, L., Wang, F., Bramanti, E., Maskow, T. & Zaray, G. 2009 *Evaluation of solvent tolerance of microorganisms by microcalorimetry*. *Chemosphere* **74**(10), 1407–1411.
- Gray, N. F. 2004 *Wastewater Treatment Biology*. Imperial College Press, London.
- Groffman, P. M., Altabet, M. A., Böhlke, J. K., Butterbach-Bahl, K., David, M. B., Firestone, M. K., Giblin, A. E., Kana, T. M., Nielsen, L. P. & Voytek, M. A. 2006 *Methods for measuring denitrification: diverse approaches to a difficult problem*. *Ecological Applications* **16**(6), 2091–2122.
- Haandel, A. C. & Lubbe, J. G. M. 2012 *Handbook of Biological Wastewater Treatment: Design and Optimisation of Activated Sludge Systems*. IWA Publishing, London.
- Hernandez-Martinez, G. R., Ortiz-Alvarez, D., Perez-Roa, M., Urbina-Suarez, N. A. & Thalasso, F. 2018 *Multiparameter analysis of activated sludge inhibition by nickel, cadmium, and cobalt*. *Journal of Hazardous Materials* **351**, 63–70.
- Inglezakis, V. J., Kudarova, A., Tarassov, D., Jetybayeva, A., Myngtay, Y., Zhalmuratova, D. & Nurmukhambetov, D. 2017a *Inhibitory effects of polar and non-polar organic substances on activated sludge activity*. *Desalination and Water Treatment* **91**, 185–191.

- Inglezakis, V. J., Malamis, S., Omirkhan, A., Nauruzbayeva, J., Makhtayeva, Z., Seidakhmetov, T. & Kudarova, A. 2017b Investigating the inhibitory effect of cyanide, phenol and 4-nitrophenol on the activated sludge process employed for the treatment of petroleum wastewater. *Journal of Environmental Management* **203**, 825–830.
- ISO-8192:2007 2007 *Water Quality – Test for Inhibition of Oxygen Consumption by Activated Sludge for Carbonaceous and Ammonium Oxidation*.
- ISO-9509:2006 2006 *Water Quality- Toxicity Test for Assessing the Inhibition of Nitrification of Activated Sludge Microorganisms*.
- ISO-9888:1999 1999 *Water Quality- Evaluation of Ultimate Aerobic Biodegradability of Organic Compounds in Aqueous Medium – Static Test (Zahn-Wellens Method)*.
- Kim, Y. M., Cho, H. U., Lee, D. S., Park, D. & Park, J. M. 2011 Comparative study of free cyanide inhibition on nitrification and denitrification in batch and continuous flow systems. *Desalination* **279**(1–3), 439–444.
- Klauson, D., Kivi, A., Kattel, E., Klein, K., Viisimaa, M., Bolobajev, J., Velling, S., Goi, A., Tenno, T. & Trapido, M. 2014 Combined process for wastewater purification: treatment of a typical landfill leachate with a combination of chemical and biological oxidation process. *Journal of Chemical Technology & Biotechnology* **90**(8), 1527–1536.
- Klauson, D., Klein, K., Kivi, A., Kattel, E., Viisimaa, M., Dulova, N., Velling, S., Trapido, M. & Tenno, T. 2015 Combined methods for the treatment of a typical hardwood soaking basin wastewater from plywood industry. *International Journal of Environmental Science and Technology* **12**(11), 3575–3586.
- Klein, K., Kattel, E., Goi, A., Kivi, A., Dulova, N., Saluste, A., Zekker, I., Trapido, M. & Tenno, T. 2017 Combined treatment of pyrogenic wastewater from oil shale retorting. *Oil Shale* **34**(1), 82–96.
- Lin, Y., Tay, J., Liu, Y. & Hung, Y. 2009 Biological nitrification and denitrification processes. *Handbook of Environmental Engineering* **8**, 539–588.
- Milenkovski, S., Bååth, E., Lindgren, P. E. & Berglund, O. 2010 Toxicity of fungicides to natural bacterial communities in wetland water and sediment measured using leucine incorporation and potential denitrification. *Ecotoxicology* **19**(2), 285–294.
- Ochoa-Herrera, V., Banihani, Q., Leon, G., Khatri, C., Field, J. A. & Sierra-Alvarez, R. 2009 Toxicity of fluoride to microorganisms in biological wastewater treatment systems. *Science of the Total Environment* **412–413**, 380–385.
- Oh, J. & Silverstein, J. 1999 Oxygen inhibition of activated sludge denitrification. *Water Research* **33**(8), 1925–1937.
- Philips, S., Laanbroek, H. J. & Verstraete, W. 2002 Origin, causes and effects of increased nitrite concentrations in aquatic environments. *Reviews Environment Science & Biotechnology* **1**(2), 115–141.
- Sibag, M., Choi, B. G., Suh, C., Lee, K. H., Lee, J. W., Maeng, S. K. & Cho, J. 2015 Inhibition of total oxygen uptake by silica nanoparticles in activated sludge. *Journal of Hazardous Materials* **283**, 841–846.
- Stasinakis, A. S., Mamais, D., Thomaidis, N. S., Danika, E., Gatidou, G. & Lekkas, T. D. 2008 Inhibitory effect of triclosan and nonylphenol on respiration rates and ammonia removal in activated sludge systems. *Ecotoxicology and Environmental Safety* **70**(2), 199–206.
- Su, Y., Zheng, X., Chen, A., Chen, Y., He, G. & Chen, H. 2015 Hydroxyl functionalization of single-walled carbon nanotubes causes inhibition to the bacterial denitrification process. *Chemical Engineering Journal* **279**, 47–55.
- Sun, F., Fan, L. & Xie, G. 2016 Effect of copper on the performance and bacterial communities of activated sludge using illumina MiSeq platforms. *Chemosphere* **156**, 212–219.
- Villemur, R. 2013 The pentachlorophenol-dehalogenating *Desulfitobacterium hafniense* strain PCP-1. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* **368**(1616), 20120319.
- Zhang, L., Zheng, P., He, Y. & Jin, R. 2009 Performance of sulfate-dependent anaerobic ammonium oxidation. *Science in China, Series B: Chemistry* **52**(1), 86–92.