Nitrification inhibition test of salty wastewater containing Tetrakishydroxymethyl phosphonium sulphate (THPS), formaldehyde and methanol using salt-adapted nitrifying bacteria, an alternative test method to ISO method

Chhetri R.K.[1,*], Kokkoli A.[1], Karvelas S.[1], Andersen H.R.[1]

[1]Department of Environmental Engineering, Technical University of Denmark, Bygningstorvet, Building 115, 2800 Kgs. Lyngby, Denmark.

*corresponding author: Ravi Kumar Chhetri, e-mail: rakc@env.dtu.dk

Abstract

Nitrification inhibition test is a standard method to test if the chemicals or samples received by wastewater treatment plants (WWTPs) are toxic to nitrifying bacteria in the wastewater treatment plant. Nitrification inhibition test based on ISO method ISO9509 or modified ISO9509 method (REFLAB method) cannot differentiate the toxicity between salt and toxicant in the sample, which is unrealistic for regulation of salty wastewaters that are treated in WWTPs with sufficient volume to dilute salts to harmless levels. To overcome the toxicity due to salinity to the nitrifying bacteria, salt-adapted nitrifying bacteria were grown on the Z400 MBBR carriers. The aim of this work was to validate and compare the nitrification inhibition of samples measured from salt-adapted nitrification inhibition test method (DTU method) with REFLAB method. The new salt-adapted nitrification inhibition test was validated by investigating the statistical uncertainty on the use of new test method with the existing REFLAB method.

The inhibition concentration of formaldehyde and methanol were similar when nitrification inhibition experiment was conducted using DTU method and REFLAB method. Lower inhibition concentration of Tetrakishydroxymethyl phosphonium sulphate (THPS) was observed with the DTU method. This means it is more sensitive to THPS and thus detects it at lower concentrations. The difference between the methods might be due to sorption of chemicals into the sludge as the biomass concentration in the DTU method is lower. The new methods standard deviation of nitrification inhibition around the 50% inhibition level was below 3%. The method is thus well suited to replace the REFLAB method for salty water samples as both repeatability and sensitivity is similar or better.

Keywords: Nitrification inhibition, wastewater, THPS, Formaldehyde, Moving bed biofilm reactor

1. Introduction

Untreated wastewater contains pollutants such as pathogens, nutrients and oxygen-demanding substances that can compromise the water quality of the receiving water bodies. In earlier years, natural treatment processes were capable of treating wastewater naturally but the industrial and population growth made the treatment of domestic wastewater of vital importance (USEPA, 2004).

Wastewater treatment plants (WWTPs) are facilities that combine various physical, chemical and biological processes able to treat industrial wastewater and remove pollutants from water (Anjum et al., 2016).

Ammonium is an oxygen demanding nutrient (up to 4.57 g O_2/g NH_4-N) and toxic to aquatic microorganisms therefore treatment of ammonium is mandatory. Nitrification is a two-step biological process able to treat ammonium. In the first step, nitrifying bacteria oxidize ammonium (NH_4-N) to nitrite (NO_2-N) whilst the second step is the oxidation of nitrite to nitrate (NO_3-N).

Nitrifying bacteria are sensitive to chemical inhibition such as a range of organic and inorganic compounds, heavy metals, chlorinated organic chemicals, low pH and high salinity (Rittmann, Bruce E.; McCarty, 2001).

Table 1. Median inhibition concentration (IC50) of chemicals obtained from nitrification inhibition tests with salt-adapted method and REFLAB method (modified ISO9509 method). Nitrification inhibition test with REFLAB method was conducted with sludge from Lundtofte WWTP. 95% confidence interval is presented in parenthesis.

Compound	IC_{50}	
	Salt-adapted method	**REFLAB method**
Formaldehyde (mg/L)	**61.2** (39.7-93.2)	**42.2** (27.8-61.4)
Methanol (mg/L)	**417** (271-629)	**225** (153-312)
THPS (mg/L)	**2.5** (2.0-2.9)	**15** (8-27)

Nitrification inhibition test is a standard method to test if the chemicals or samples received by wastewater treatment plant are toxic to the nitrifying bacteria in the wastewater treatment plant. Existing nitrification inhibition tests such as ISO 9509 (2006) or modified ISO 9509 method (REFLAB method, (2004)) cannot differentiate the toxicity between salt and toxicant in the sample, which is unrealistic for regulation of wastewaters with high salinity treated in WWTPs with sufficient volume to dilute salts to harmless levels. Therefore new test method on a nitrification inhibition was developed based on salt-adapted nitrifying bacteria grown on the Z400 MBBR carriers. The advantage of this method is that the nitrifying bacteria are able to withstand saline levels that would be

toxic to conventional activated sludge used in the existing nitrification inhibition tests. The aim of this paper was to validate and compare the nitrification inhibition of samples tested with new salt-adapted nitrification test (DTU method) with ISO 9509 and REFLAB method. Three chemicals namely, Tetrakishydroxymethyl phosphonium sulphate (THPS), formaldehyde and methanol were spiked separately to the saline wastewater to test the nitrification inhibition using three test methods.

2. Materials and Methods

A salt adapted nitrification bacteria was cultured in a 9 L flow through reactor in the Department of Environmental Engineering at Technical University of Denmark. The bioreactor was started by adding 3 L activated sludge obtained from Lundtofte WWTP, 1000 carriers (Z400) provided from AnoxKaldnes AB, Sweden and saline water with a target concentration of 40 g NaCl/L. Bioreactor was fed with 2 feed bottles containing ammonium bicarbonate, sodium bicarbonate and trace chemicals (Van De Graaf et al., 1996). Salinity in the feed bottle 1 with ammonium bicarbonate and sodium bicarbonate was maintained by adding 40 g NaCl/L and salinity in feed bottle 2 with trace chemicals was maintained by adding sea salt. The pH was maintained between 7.5 and 7.9 and conductivity was maintained at 50.5±1 mS/cm. Saline wastewater was collected from the oil separation facility in Denmark and treated using laboratory scale three staged moving bed biofilm reactors (MBBR). Effluent from MBBR reactors was used to spike the chemicals for nitrification inhibition experiments.

THPS was quantified with iodine titration by using the Tolcide PS chemical test kit, #4-8776-01, LaMotte. Formaldehyde was measured by a formaldehyde reagent test set, 3-500 µg/L CH2O, #2257700, Hach Lange. Methanol was quantified by using GC-MS. The determination of total suspended solids (TSS) was made following the standard method (APHA, 2012). The analysis of ammonia, nitrate, nitrite and phosphate was carried out using the auto-sampler San ++, SKALAR.

The nitrification inhibition of different concentrations of THPS, formaldehyde and methanol were tested using DTU method and REFLAB method. In short, nitrification inhibition test was done in a 500 mL batch reactor. For DTU method, 50 carriers with optimally grown culture were transferred from main bioreactor. For REFLAB method, 2 g/L activated sludge was added in the batch reactor. Nitrification (degradation of ammonium and formation of nitrate and nitrite) was studied for 4 hours in batch reactors. pH, conductivity and oxygen in the batch reactors were recorded at the beginning and end of the nitrification inhibition test.

Normalized ammonium degradation rate constant (k) of sample and different concentration of chemicals was plotted using sigmoidal dose-response curve to calculate the median inhibition concentration (IC_{50}). To verify the IC_{50} values of chemicals from the DTU method and REFLAB method were statistically significant, student t–test was done.

3. Results and Discussion

Median inhibition concentration (IC_{50}) of each chemicals with 95% confidence interval of chemicals obtained from two test methods is presented in the Table 1. Nitrifying bacteria are sensitive to THPS compare to formaldehyde and methanol when tested with both test methods on nitrification inhibition.

The statistical analysis from the student t- test, presented in

Table *2*., showed that the difference of IC_{50} of formaldehyde and methanol between the two methods was not significant. For the THPS, the IC_{50} values obtained from two methods vary in a greater degree. A possible reason for the difference in the IC_{50} values could be due to the bio-sorption of the chemicals into the sludge. It is very important that all significant deviation in sensitivity is towards better sensitivity of the DTU method which ensure that replacing the REFLAB method will not results in a lower ability to detect nitrification inhibition in the water so the protection of the wastewater treatment function will not be reduced.

To test inter- and intra-day reproducibility of the DTU method, IC50 of formaldehyde (61 mg/L) was spiked to the effluent from saline MBBR reactors to obtain 50% nitrification inhibition. For inter-day reproducibility nitrification inhibition of chemical spiked MBBR effluent was measured on five replicates and for intraday reproducibility nitrification inhibition was measured in five different days. The nitrification inhibition percentages of all the replicates over the 5 days were quite similar with 23 out of the 25 samples in the range of 41-49% inhibition. The standard deviation (s_M) of DTU method on nitrification inhibition of formaldehyde spiked MBBR effluent was 2.97% and standard error on mean (S.EM) was 0.6%. Thus, DTU method is suitable to test the nitrification inhibition of saline wastewater since it differentiate the toxicity between salt and actual toxicants.

Overall DTU method can be used as a standard method for nitrification inhibition test of saline wastewater. The IC_{50} values of formaldehyde and methanol between DTU method and REFLAB method were similar. In terms of THPS, DTU method showed higher sensitivity probably due to the bio-sorption of the chemical in the activated sludge. DTU method offers robustness and it is easy to implement. The main advantage is that the nitrifying bacteria withstand the toxicity due to high salinity therefore it is feasible to differentiate the toxicity between salt and toxicant in the sample. Finally, inter- and intra-day results showed that the repeatability of the test resulted in similar results and thus it is suitable to be used for nitrification inhibition of saline wastewater.

References

Anjum, M., Al-Makishah, N.H., Barakat, M.A., 2016. Wastewater sludge stabilization using pre-treatment methods. Process Saf. Environ. Prot. 102, 615–632. https://doi.org/10.1016/j.psep.2016.05.022

APHA, 2012. Standard Methods for the Examination of water and wastewater (22nd Edition). American Public Health Association /American Water Works Association/Water Environment Federation, Washington DC, USA.

ISO 9509, 2006. ISO 9509:2006 - Water quality - Toxicity test for assessing the inhibition of nitrification of activated sludge microorganisms.

REFLAB method, 2004. Nitrifikationshaemning for kemikalier og spildevand Bestemmelse med aktiveret slam Method for assessing the inhibition of nitrification of activated sludge micro-organisms by chemicals and waste water.

Rittmann, Bruce E.; McCarty, P.L., 2001. Environmental Biotechnology: Principles and Applications. McGraw-Hill Ser. Water Resour. Environ. Eng. https://doi.org/10.1007/978-3-540-88546-7_72

USEPA, 2004. Primer for Municipal Wastewater Treatment Systems.

Van De Graaf, A.A., De Bruijn, P., Robertson, L.A., Jetten, M. 5 M., Gijs Kuenen, J., 1996. Autotrophic growth of anaerobic ammonium-oxidizing microorganisms in a fluidized bed reactor.

Table 2. Statistical summary of t-test of IC_{50} of chemicals and mixture of chemicals obtained from nitrification inhibition test from salt-adapted method and REFLAB method (tested with activated sludge from Lundtofte WWTP)

Compound	(IC_{50}) DTU method	(IC_{50}) REFLAB method	t	df	P value	Significant (α=0.05)
Formaldehyde	61	42	1.5	10	0.155	No
Methanol	417	225	2	12	0.071	No
THPS	2.5	15	3.6	11	0.004	Yes