Nitrous oxide formation during nitritation and nitrification of high-strength wastewater

SUPPORTING INFORMATION

DESCRIPTION OF ADDITIONAL EXPERIMENTS FOR QUANTIFICATION OF DENITRIFICATION TO N_2O FORMATION

Additional short-term experiments were conducted to investigate the contribution of denitrification to N_2O formation. In these tests, AOB were inhibited by addition of allylthiourea.

Materials and methods

A volume of 1.5 L of mixed liquor was removed from the reactor (in nitrification mode) as inoculum for additional inhibition tests. These were performed in a smaller chemostat reactor with 1.5 L operating volume. The reactor was equipped with magnetic stirring and fine bubble aeration. It was fed from the same storage tank as the source reactor at a rate of 0.5 mL/min in order to achieve the same dilution rate as in the original scale. Temperature, pH, DO and dissolved N_2O concentration were measured and recorded online with equipment comparable to the source reactor.

At the beginning of the test, allylthiourea was added to the reactor at a concentration of 20 mg/L, which was double the concentration reported to lead to complete inhibition of AOB activity by Ginestet *et al.* (1998) in order to make up for dilution with inflow during the test. Allylthiourea selectively inhibits AOB activity (ammonia monooxygenase inhibition), but not NOB or heterotrophic bacteria (Ginestet *et al.* 1998). Nitrite was added up to a concentration of 100 mg NO_2^--N/L. After starting the inflow, the reactor was aerated until N_2O was completely removed from the liquid phase and DO concentration reached 5 mg O_2/L. Aeration was then turned off and DO as well as dissolved N_2O concentration were followed for 15 min. This was repeated twice with fresh inoculum and with lower DO concentration of 2 mg O_2/L, respectively.

doi: 10.2166/wst.2013.144supplement

The concentration of ammonium, nitrite and nitrate was measured in the inoculum prior to the test, in the inflow and effluent of the reactor.

Results

As all repetitions yielded comparable results, the values for one test with 5 mg O_2/L are presented here and can be considered representative for all tests. The pH was 6.5 at the beginning of the tests and increased slightly up to 6.7 due to the higher pH of the inflow (7.7). Temperature was stable around 25 °C. The concentration of nitrogen compounds is compiled in Table S-1. The difference between inflow and effluent concentrations could be attributed completely to the dilution effect inside the reactor. No significant nitrogen conversion could be determined.

The concentration profiles obtained from DO measurement showed stable values, apart from normal background scattering. Similarly, the concentration of dissolved N_2O was stable throughout the test; there was some background scattering but no significant trend in concentration changes. The concentration profiles of DO and N_2O are depicted in Figure S-1.

Discussion

The DO curves of all tests indicated that no oxygen was consumed during the test. Similarly, no significant changes in the concentration of dissolved N_2O were observed in any of the tests. In contrast, the OUR of the source reactor

Table S-1 | Results of nitrogen concentration (c) measurements

	Reactor beginning	Inflow	Effluent after 15 min
c(NH_4^+-N) [mg/L]	220	265	226
c(NO_2^--N/L) [mg/L]	102	<0.6	95.4
c (NO_3^--N/L) [mg/L]	123	<0.6	117.6

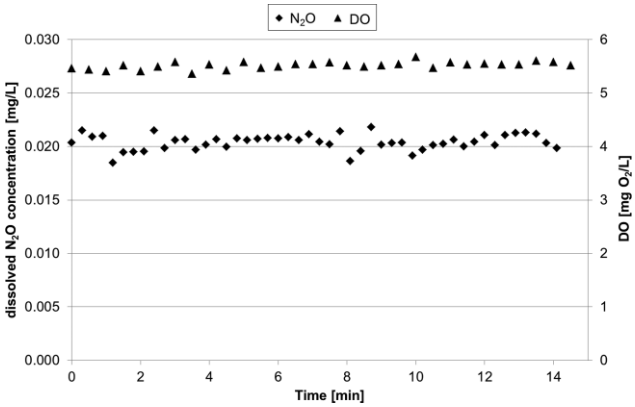

Figure S-1 | Development of dissolved N_2O concentration and DO during the inhibition test.

when the inoculum mixed liquor samples were derived was about 4 mg $O_2/(L \cdot h)$. The current N_2O formation rate in the source reactor was 0.07 mg $N_2O/(L \cdot h)$.

Therefore, it can be concluded that AOB were completely inhibited and heterotrophic oxygen consumption was also insignificant. NOB activity was probably inhibited by sudden exposure to elevated nitrous acid concentration (0.23 mg HNO_2/L). Similarly, the stable concentration of N_2O implies that upon inhibition of AOB (ammonium conversion activity) none of the present bacteria exhibited any N_2O formation activity. Accordingly, significant contribution of heterotrophic (denitrifying) bacteria to N_2O formation in the source reactor can be excluded.

REFERENCE

Ginestet, P., Audic, J.-M., Urbain, V. & Block, J.-C. 1998 Estimation of nitrifying bacterial activities by measuring oxygen uptake in the presence of the metabolic inhibitors allylthiourea and azide. *Appl. Environ. Microbiol.* **64**, 2266–2268.