Determination of optimal dose of allylthiourea (ATU) for the batch respirometric test of activated sludge

Bing Liu, Mitsuharu Terashima, Nguyen Truong Quan, Nguyen Thi Ha, Le Van Chieu, Rajeev Goel and Hidenari Yasui

ABSTRACT

Allylthiourea is a known specific inhibitor for ammonium oxidiser to suppress its oxygen uptake, and is commonly used for various kinds of batch respirometric tests to detect heterotrophic respiration in activated sludge. However, when high heterotrophs were present in the sample, it appeared the inhibitor was noticeably degraded and reached below the inhibition threshold after a couple of days, which resulted in overestimation of the heterotrophic respiration. The biological decomposition of the inhibitor was expressed with a Monod-type rate expression having a half-saturation coefficient of 980 mg-COD/L and maximum specific growth rate of 1.0 d^{-1}. The developed kinetic model, including the growth and decay of the heterotrophs and nitrifiers, indicated that the ATU with about 90 mg-ATU/L which was initially dosed to the system would reach below the inhibition threshold of 1.0 mg-ATU/L after 10 days when 750 mg-COD/L of heterotrophs were present. From the kinetic model, an empirical formula to calculate a safe minimum ATU dose for the batch respirometric test was elaborated. The model also provided a modified experimental procedure to accurately estimate the initial heterotrophic biomass concentration in the sample and its specific decay rate based on IWA Activated Sludge Models.

Key words | biodegradation of inhibitor, kinetic model, oxygen uptake, safe minimum ATU dose

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INTRODUCTION

The respirometer is an automated logging instrument to record microbial oxygen uptake in a batch mode, and is widely used to monitor endogenous oxygen uptake of microorganisms as well as evaluation of biological degradability of various kinds of substrates (Cherif *et al.* 2016; Ferro Orozco *et al.* 2016; Mora *et al.* 2016; Paździor *et al.* 2016; Vitanza *et al.* 2016; Wan *et al.* 2016; Fall & Silva-Hernandez 2017). In principle, the instrument is composed of a microprocessor for data logging and a set of closed incubation vessels equipped with a precious gas pressure sensor and/or DO probe to detect the decrease of O$_2$ partial pressure in the head-space. When a certain amount of DO is consumed in the incubation vessel, O$_2$ is automatically supplied from an external oxygen gas cylinder in order to maintain high DO. As a respirometer enables monitoring of the oxygen uptake with very short intervals, e.g. every 1 minute, this method is also applied to estimate the kinetics of readily slowly-biodegradable materials in the sample that are

oxidised in the initial phase of the experiment. At present, the respirometer is supposed to be an essential tool to measure organic fractions in the activated sludge such as active heterotrophic organism (OHO), storage compounds and slowly hydrolysable organic particulates.

With respect to the estimation of the OHO fraction in activated sludge, the endogenous oxygen uptake rate (OUR$_e$) of the microorganism is usually focused on (Ekama *et al.* 1986). From the OUR$_e$ plot over time (respirogram), the initial OHO concentration in the sample (X$_{OHO(0)}$) and its specific decay rate (b$_{OHO}$) are mathematically calculated based on the Activated Sludge Model concept (Henze *et al.* 2000). On the other hand, the off-line respirometry technique is also available in laboratories as a simple and inexpensive experimental method, where the activated sludge in an aerated open incubation vessel is manually sampled and transferred into another small closed vessel such as a Winkler bottle at regular intervals, and its OUR$_e$ is measured with

DO meter. Since the method requires manual operations, this is rather cumbersome and therefore the number of data plots per experiment is limited. This may result in lower analytical accuracy in terms of parameter estimation than those obtained from the automated on-line respirometer.

In order to measure $X_{OHO(0)}$ and b_{OHO}, both of the above experimental methods require suppression of oxygen uptake from nitrifiers, since the ammonium oxidation also consumes oxygen. For this purpose, allylthiourea (ATU) is commonly used to inhibit the reaction (Bedard & Knowles 1989; Dapena-Mora *et al.* 2007; Lu *et al.* 2010; Zhou & Oleszkiewicz 2010; Jäntti *et al.* (2013); Rattier *et al.* 2014; Krustok *et al.* 2016; Men *et al.* 2017; Tatari *et al.* 2017; Wang *et al.* 2017; Zhang *et al.* 2017). When an ammonium oxidising organism (AOO) is inhibited by ATU, oxygen uptake of the nitrite oxidising organism (NOO) is also minimised due to limitation of the nitrite supply from AOO. For measuring carbonaceous BOD for 5 days in a Winkler bottle with diluted sample water, the ATU dose has been standardised to be 20 mg/L in order to suppress the nitrification during the batch incubation test (Reuschenbach *et al.* 2003).

However, Friedrich *et al.* (2017) recently pointed out that a reliable procedure to determine a safe ATU dose for batch respirometric tests was still lacking and not explicitly mentioned in literature. This is a noticeable criticism for the respirometry methods. Since the methods are usually conducted under high OHO, if the high OHO decomposes ATU significantly, the ATU concentration will be eventually reduced below its inhibition threshold against nitrification after a certain period of time. To clarify this, a set of batch tests under differentiated ATU dose was conducted in this study. During the experiment, the ATU concentrations in the incubation vessels were regularly measured and the recovery of nitrification was monitored. From the experiment and Activated Sludge Model concept (Henze *et al.* 2000), a procedure to determine a safe minimum ATU dose is discussed in this paper.

MATERIALS AND METHODS

Respirometric test

Fresh nitrifying activated sludge was collected from a low-loaded municipal wastewater treatment plant (sludge retention time = *ca.* 15 day), Kitakyushu, Japan, and centrifugally washed twice using 10 mmol/L phosphate buffer (Na_2HPO_4 and KH_2PO_4, pH = 7.0). After the washing, NH_4Cl solution was mixed with the activated sludge (1,500 mg COD/L) to be 100 mg N/L as the substrate for the nitrifier. The mixture was then placed in gas-tight 500-mL medium bottles with initial ATU concentrations at 0, 20, 50 and 100 mg/L respectively (Wako Chemical, Japan). The bottles were set up in a commercial respirometer (AER-800, Challenge Technology, USA). The OURs were logged at 2-hour interval for 10 days under $25 \pm 0.2\,^\circ C$ in a temperature-controlled chamber (FMC-1000, EYELA, Japan). After completion of the experiment, the activated sludge was again sampled from the same wastewater treatment plant, and the second experiment was identically repeated. A total of eight OUR datasets with four kinds of ATU dose was obtained accordingly.

Measurement of ATU and other analytical methods

To measure ATU concentration in the above batch experiments, an identical set of the respirometry vessels was prepared in the second experiment, except that the vessels were opened to the air. About 10 mL of the liquid per vessel was collected at about 8-hour intervals in a day, and immediately filtered. The ATU concentration of the filtrate was analysed within a couple of hours using an ultra-performance liquid chromatography system equipped with a refractive index detector (Acquity UPLC system, Waters, USA) and an ODS-column (Acquity HSS T3, 1.8 μm, 2.1 mm × 100 mm, Waters, USA). The eluant, composed of acetonitrile (10%) and ultra-pure water (90%), was fed to the ODS-column at 0.2 mL/min of constant flow rate where the system temperature was kept constant at 40 °C. The detectable limit of ATU by the instrument was about 0.2–0.5 mg/L. Concentrations for the activated sludge COD, ammonium-N, nitrite-N and nitrate-N were obtained according to *Standard Methods* (APHA/AWWA/WEF 2012).

Some kinetic and stoichiometry coefficients

The parameters of ATU biodegradation by OHO: From the datasets of the respirograms and the decrease of ATU concentrations along with time, the maximum specific growth rate from ATU ($\mu_{max\_ATU,\ OHO}$) and the half-saturation coefficient of ATU ($K_{S\_ATU,\ OHO}$) were determined assuming the biological reaction rate was expressed with Monod-equation whilst the OHO yield coefficient from ATU (Y_{OHO_ATU}) was also obtained from the increment of oxygen uptake between the system fed with ATU and that without addition of ATU, which was approximated to be $(1-Y_{OHO\_ATU}) \times$ COD-based ATU.

The parameters of endogenous oxygen uptake by OHO: In order to ensure the detection of endogenous oxygen

uptake from OHO in the activated sludge sample, a manual off-line respirometry with addition of ATU was also simultaneously conducted. About 2L of the above conditioned activated sludge sample was aerated in an open flask for 10 days, and sampled into a 100-mL Winkler bottle every day. After 15 minutes from the addition of ATU at 20 mg/L, the heterotrophic OUR_e was measured with a DO meter (TOX-999B, Toko, Japan). Based on the respirogram for 10 days, $X_{OHO(0)}$ and b_{OHO} were calculated according to Equation (1) (Henze *et al.* 2000). The parameters were also used to check the consistency with those obtained from the automated on-line respirometer.

$$\begin{cases} OUR_{e(t)} = b'_{OHO} \cdot (1 - f'_U) \cdot X_{OHO(0)} \cdot \exp(-b'_{OHO} \cdot t) \\ b'_{OHO} = (1 - Y_{OHO\_SB})(1 - f_U)b_{OHO} \\ f'_U = \dfrac{1}{(1 - Y)(1 - f_U)}f_U \end{cases} \quad (1)$$

where, $OUR_{e(t)}$: endogenous oxygen uptake rate of OHO at day = t (mg-O_2/L/d), b_{OHO}: specific endogenous decay rate of OHO (day^{-1}), b'_{OHO}: apparent specific endogenous decay rate of OHO (day^{-1}), f_U: fraction of biomass leading to particulate organic inert product (0.08 g-COD/g-COD), t: incubation time (day), Y_{OHO_SB}: OHO yield coefficient of the cryptic growth from the biodegradable organics, S_B generated by the endogenous decay (0.63 g-COD/g-COD), $X_{OHO(0)}$: initial OHO concentration in the activated sludge (mg-COD/L).

The parameters of nitrification by nitrifiers: To estimate maximum specific growth rate (μ_{max_AOO}), the yield coefficient (Y_{AOO_NHx}), and initial AOO concentration ($X_{AOO(0)}$) in the activated sludge sample, another batch incubation test was carried out where 75 mg COD/L of the activated sludge sample was seeded in a 500 mL aerated flask equipped with a pH controller. In the flask, 50 mg N/L of ammonium nitrogen (as NH_4Cl) was maintained within ± 10% accuracy throughout the incubation period for 30 days using a computer-aided syringe pump (SP-2PC, AS ONE, Japan) where high-strength NH_4Cl solution (20,000 mg N/L) was continuously injected to the flask. To programme the injection speed of the syringe pump, the ammonium nitrogen concentration in the flask was manually monitored at 0.5 day

intervals. When the ammonium nitrogen concentration was out of the pre-determined range (47–53 mg N/L) at the next monitoring event, the injection speed was slightly adjusted to meet the nitrification rate of the flask. Adopting a specific decay rate (b_{AOO}) and half-saturation coefficient (K_{S,AOO_NHx}) of AOO from literature (Henze *et al.* 2000; Makinia 2010), the parameters were calibrated from the exponential elevation of the OUR along with time according to Equation (2). Similarly, the parameters for NOO (Y_{NOO_NO2}, μ_{max_NOO} and $X_{NOO(0)}$) were also obtained using 50 mg N/L of $NaNO_2$ for NOO's substrate. The ATU inhibition for AOO in the activated sludge sample was also checked in a preliminary batch experiment with addition of 50 mg N/L of ammonium-N. The experiment confirmed that about 0.5–1.0 mg/L of ATU (0.84–1.7 mg COD/L) was enough to suppress the ammonium oxidation.

$$\begin{cases} OUR_{N(t)} = \left(\dfrac{3.43 - Y_{AOO\_NHx}}{Y_{AOO\_NHx}}\right)X_{AOO(0)} \cdot \exp(\mu_{AOO} \cdot t) + (1 - f_U)b_{AOO} \cdot X_{AOO(0)} \\ \mu_{AOO} = \mu_{max\_AOO,NHx}\left(\dfrac{S_{NHx}}{K_{S,AOO\_NHx} + S_{NHx}}\right) \end{cases} \quad (2)$$

where, $OUR_{N(t)}$: nitrogenous oxygen uptake rate of AOO at day = t (mg-O_2/L/d), 3.43: coefficient (g-COD/g-N), b_{AOO}: specific endogenous decay rate of AOO (day^{-1}), f_U: fraction of biomass leading to particulate organic inert product (0.08 g-COD/g-COD), K_{S,AOO_NHx}: half-saturation coefficient of ammonium-N on AOO growth (mg-N/L), t: incubation time (day), $X_{AOO(0)}$: initial AOO concentration in the activated sludge (mg-COD/L), Y_{AOO_NHx}: AOO yield coefficient from ammonium-N (g-COD/g-N), μ_{AOO}: specific growth rate of AOO (day^{-1}), $\mu_{max\_AOO,NHx}$: maximum specific growth rate of AOO (day^{-1}).

Kinetic modelling

Based on the IWA Activated Sludge Model No. 1 (Henze *et al.* 2000) with a modification in a two-step nitrification, the above monitored concentrations and OURs were dynamically simulated using a process simulator (GPS-X 6.4, Hydromantis Software Inc., Canada). In order to express the strong inhibition of ATU against AOO, as shown in Equation (3), a power coefficient n (≥ 1) was applied to the conventional non-competitive inhibition switching function, I_{ATU}. When big n was chosen (e.g. $n = 5$, I_{ATU} sharply responded from 1 to/from zero around $K_{I,\ ATU}$. This $K_{I,}$

ATU was defined as the ATU inhibition threshold for AOO.

$$
\begin{cases}
\mu_{AOO} = \mu_{max\_AOO,NHx} \left(\dfrac{S_{NHx}}{K_{S,NHx} + S_{NHx}} \right) \times I_{ATU} \\[2mm]
I_{ATU} = \left(\dfrac{K_{I,ATU}^n}{K_{I,ATU}^n + S_{ATU}^n} \right)
\end{cases}
\tag{3}
$$

where, μ_{AOO}: specific growth rate of AOO (day^{-1}), $\mu_{max\_AOO,NHx}$: maximum specific growth rate of AOO (day^{-1}), $K_{S,NHx}$: half-saturation coefficient of ammonium-N (mg-N/L), S_{NHx}: ammonium-N concentration (mg-N/L), $K_{I,ATU}$: half-inhibition saturation coefficient of ATU (mg-COD/L), S_{ATU}: ATU concentration (mg-COD/L), n: power coefficient (-).

RESULTS AND DISCUSSION

Duration of ATU action to suppress nitrification

As shown in Figure 1(a) where no ATU was added to the vessel, the initial ammonium-N (100 mg-N/L) immediately disappeared within 1–2 days and quickly oxidised to nitrate-N, followed by a slight increase of nitrate concentration over time due to nitrification of the nitrogenous compound released

from the endogenous decay of OHO. When 20 mg/L of ATU was dosed (Figure 1(b)), the ATU could suppress the nitrification until about day 4. During the period, ammonium-N in the vessel was kept increased according to the ammonification from the decayed product of OHO whilst the ATU concentration consistently decreased. After reaching an ATU concentration of about 0.5–1.0 mg/L at day 4, a sharp drop of ammonium-N concentration and corresponding nitrate accumulation were recognised, which indicated a recovery of nitrification due to the loss of ATU inhibition in the system. When 50 mg/L of ATU was dosed (Figure 1(c)), the initiation of nitrate production delayed to day 6–7 showing the ATU addition could suppress the nitrification for 6–7 days. In the experiment, the nitrate production rate after the day was slightly lower than that observed under the experiment with addition of ATU at 20 mg/L. This low nitrate production rate was attributed to the decrease of AOO biomass due to AOO's endogenous decay without growth until the day. In order to maintain the suppression of the nitrification for 10 days (duration of ATU action = 10 days), as shown in Figure 1(d), it seemed that 100 mg/L of ATU was enough to dose to the vessel at the start-up of the experiment. In the vessel, more than 10 mg/L of ATU was retained until day 3. At the end of the experiment (day 10), the residual ATU concentration was still higher than
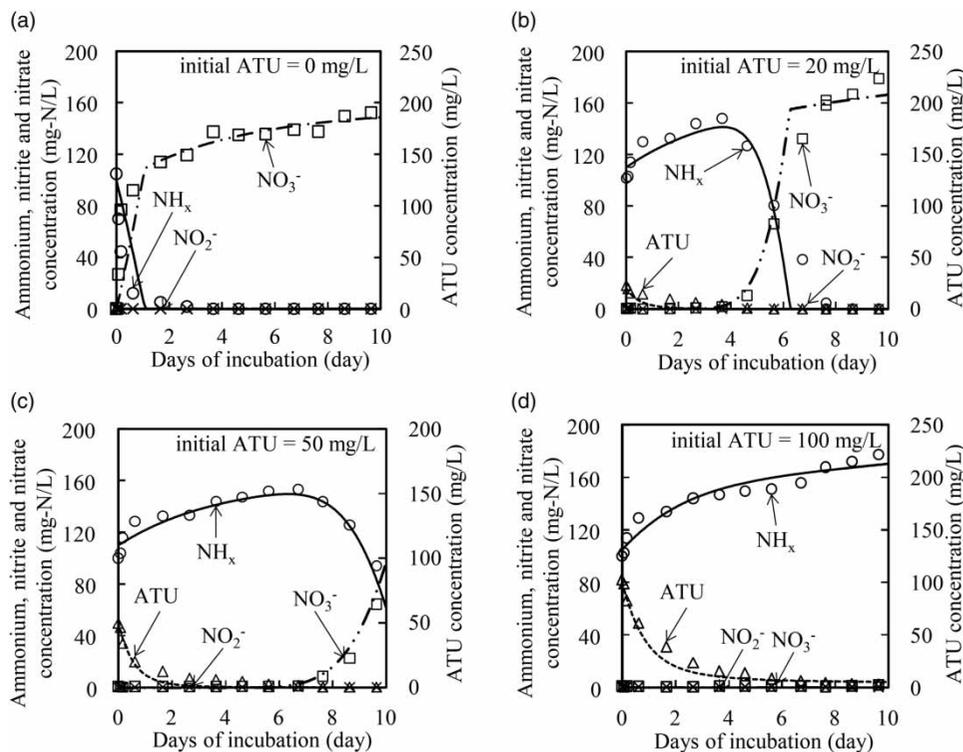

Figure 1 │ Responses of nitrification and ATU concentrations in the batch tests. (○: Ammonium-N concentration (mg-N/L), ×: Nitrite-N concentration (mg-N/L), □: Nitrate-N concentration (mg-N/L), △: ATU concentration (mg-ATU/L), plots: experimental data, lines: simulation from the kinetic model).

the inhibition threshold of nitrification. Throughout the experiments, trace nitrite-N was detected in all vessels. Therefore, to check ATU inhibition in the system, nitrate-N could be used as a monitoring parameter rather than nitrite-N.

The dynamic simulation curve for each substance was also drawn in Figure 1(a)–1(d). The concentrations of ATU, ammonium-N (NH_x), nitrite-N (NO_2^-) and nitrate-N (NO_3^-) could be fairly reproduced with the kinetic model except the experimental plots of ammonium-N and nitrate-N at day 7 in Figure 1(b). Although the exact reason for the mismatch was not clear, it was speculated that the actual I_{ATU} might be more complex than that expressed as Equation (3).

Simulation of OUR with and without ATU addition

The kinetic model could also dynamically simulate the OUR data plots of the experiments. As shown in Figure 2(a) (without addition of ATU), very high OUR was created between $t = 0$ and $t = 1$, which was derived from the rapid oxidation of the initially fed ammonium-N. The low OUR in the latter phase was expressed to be a composite of oxygen uptake consisting of the respiration of nitrifiers and the endogenous respiration of OHO. When 20 mg/L of ATU was dosed (Figure 2(b)), the OUR consistently decreased until day 2 followed by a sudden elevation due to the recovery of nitrification. At around day 3–4, the OUR peaked and nitrification was almost perfectly recovered due to low ATU in the vessel. After the peak, the OUR again decreased because of the shortage of N-source for the nitrification. Similar to the experimental result, the OUR for the vessel dosed with 50 mg/L of ATU (Figure 2(c)) showed a peak at day 8, which was also from the recovery of the nitrification. The peak was delayed by about 4 days comparing to the experiment dosed with 20 mg/L of ATU. Due to decrease of nitrifier biomass during the initial phase because of no growth, the peak height at day 8 was slightly lower than that obtained in the vessel dosed with 20 mg/L of ATU. When 100 mg/L of ATU was added (Figure 2(d)), the OUR consistently decreased along with time, and no intermediate peak was observed. However, it was noted that the shape of the OUR curve was composite and originated from the growth of OHO from ATU and the endogenous
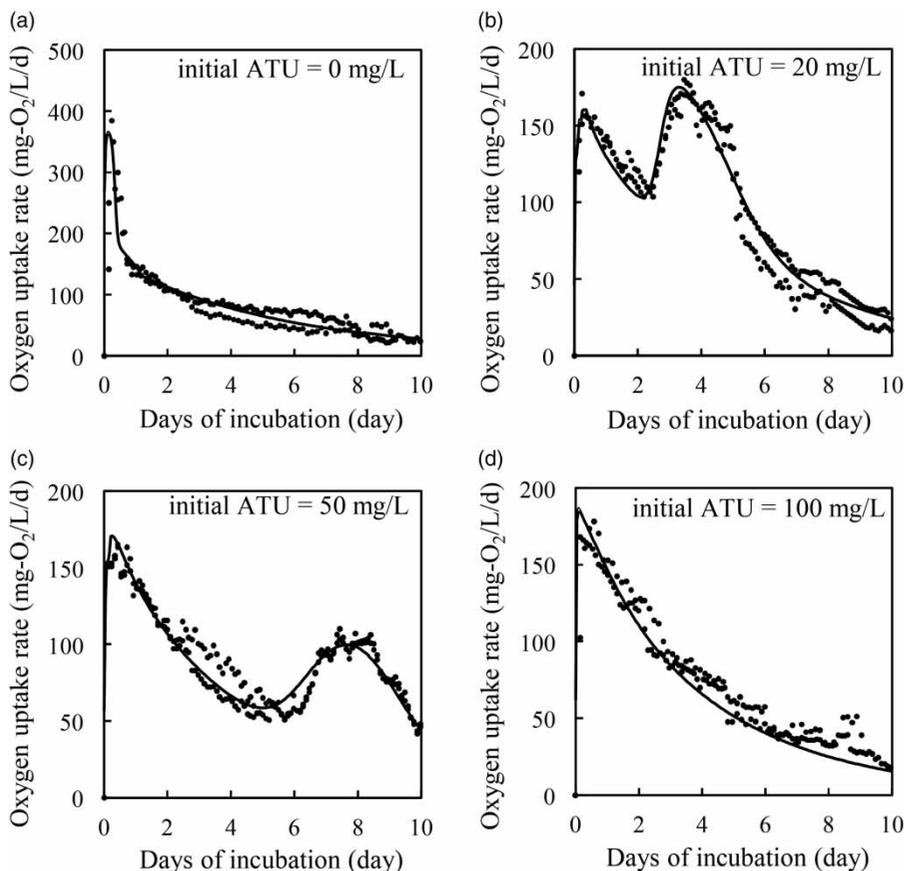

Figure 2 | Responses for oxygen uptake rate in the batch experiment (plots: experimental data, lines: simulation).

decay of OHO. As high ATU remained until about day 2–3 in the vessel, although the entire curve shape looked smooth, the slope during the initial phase and the intersection of the respirogram were noticeably affected by the biodegradation of ATU, whereas the slope in the latter phase (e.g. after day 4) was mostly dominated by the endogenous decay of OHO. Hence, careful analysis of the respirogram was necessary when b_{OHO} and $X_{OHO(0)}$ were estimated from the OUR_e with high ATU dose.

The parameters for the growth and decay of OHO, AOO and NOO in the experiments at 25 °C were summarised and are listed in Table 1. The heterotrophic reaction rate of ATU was expressed by a Monod-type rate equation with $1.0\ d^{-1}$ of $\mu_{max,\ OHO\_ATU}$, 980 mg-COD/L of $K_{S,\ OHO\_ATU}$ and 0.63 g-COD/g-COD of $Y_{OHO,\ ATU}$. Compared to ordinary soluble readily biodegradable organics for OHO in municipal wastewater (S_B), ATU seemed to give the OHO low maximum specific growth rate and high half-saturation coefficient

(e.g. $\mu_{max,\ OHO\_SB} \cong 8.5\ d^{-1}$ at 25 °C, $K_{S,\ OHO\_SB} = 20$ mg-COD/L) (Henze *et al.* 2000). If high S_B was present together with ATU in the activated sludge sample, the OHO should uptake the both depending on each substrate affinity. However, such substrate competition between S_B and ATU was not experimentally investigated since the concentration of the readily biodegradable organics generated from the biomass decay was supposed to be very limited. Therefore the OHO's uptake reactions for the 2 kinds of substrates (S_B and ATU) were modelled in a simplified form and assumed to be proportional to the availability of each substrate in this study, as shown in Equation (4).

$$\begin{cases} \text{OHO biomass to uptatke ATU} = X_{OHO} \times \left(\dfrac{S_{ATU}}{S_{ATU} + S_B} \right) \\ \text{OHO biomass to uptatke } S_B = X_{OHO} \times \left(\dfrac{S_B}{S_{ATU} + S_B} \right) \end{cases}$$
(4)

Table 1 │ Parameters for growth and decay of OHO, AOO and NOO at 25 °C

Parameter			Expression
$\mu_{max,\ OHO\_ATU}$	1.0	d^{-1}	Maximum specific growth rate of OHO from ATU
$K_{S,\ OHO\_ATU}$	980	mg-COD/L	Half-saturation coefficient of ATU on OHO growth
$\mu_{max,\ OHO\_SB}$	8.5	d^{-1}	Maximum specific growth rate of OHO from cryptic substrate, S_B[a,b]
$K_{S,\ OHO\_SB}$	20	mg-COD/L	Half-saturation coefficient of cryptic substrate, S_B on OHO growth[a]
b_{OHO}	0.71	d^{-1}	Specific endogenous decay rate of OHO[b]
$k_{max,\ OHO\_XCB}$	3.0	d^{-1}	Maximum specific hydrolysis rate of OHO for decayed product, XC_B[a]
$K_{H,\ OHO\_XCB}$	0.1	–	Half-saturation coefficient of OHO for decayed product, XC_B[a]
$Y_{OHO,\ SB}$	0.63	g-COD/g-COD	Yield of OHO from from cryptic substrate, S_B[a]
$Y_{OHO,\ ATU}$	0.63	g-COD/g-COD	Yield of OHO from ATU
$\mu_{max,\ AOO}$	0.71	d^{-1}	Maximum specific growth rate of AOO
$K_{S,\ AOO\_NHx}$	2.0	mg-N/L	Half-saturation coefficient of ammonium-N on AOO growth[a]
b_{AOO}	0.03	d^{-1}	Specific endogenous decay rate of AOO[c]
Y_{AOO_NHx}	0.208	g-COD/g-N	Yield of AOO from ammonium-N
$K_{I,\ AOO\_ATU}$	1.5	mg-COD/L	Half-inhibition saturation coefficient of ATU for AOO
n	5	–	Power coefficient of ATU inhibition for AOO
$\mu_{max,\ NOO}$	0.20	d^{-1}	Maximum specific growth rate of NOO
$K_{S,\ NOO\_NO2}$	0.20	mg-N/L	Half-saturation coefficient of nitrite-N on NOO growth[c]
b_{NOO}	0.03	d^{-1}	Specific endogenous decay rate of NOO[c]
$Y_{NOO,\ NO2}$	0.029	g-COD/g-N	Yield of NOO from nitrite-N
f_U	0.08	g-COD/g-COD	Fraction of biomass leading to particulate organic inert product
f_{XBN}	0.086	g-N/g-COD	Nitrogen content of active biomass[a]
$X_{OHO(0)}$	750	mg-COD/L	Initial biomass of ordinary heterotrophic organism
$X_{AOO(0)}$	80	mg-COD/L	Initial biomass of ammonium oxidising organism
$X_{NOO(0)}$	70	mg-COD/L	Initial biomass of nitrite oxidising organism

[a]Adopted from Henze *et al.* (2000); [b]temperature coefficient 1.035 adopted from Henze *et al.* (2000); [c]adopted from Makinia (2010).

Development of empirical equation to obtain the safe minimum ATU dose

As the above developed kinetic model enabled calculation of the decrease of ATU concentration along with time, the duration of action for the ATU inhibition was simulated by changing the initial ATU dose and initial OHO concentration respectively. For the simulation, considering a safety margin, the duration of ATU action was defined to be the time where ATU concentration reached 0.5 mg/L (0.8 mg-COD/L), which was slightly conservative compared to the calibrated $K_{I, \ AOO\_ATU}$ (1.5 mg-COD/L). As shown in Figure 3(a), the calculated duration of ATU action (plot) was extended when high ATU was dosed. In the case where initial OHO concentration was set to 1,500 mg-COD/L in the system (O), dosing 100 mg/L of ATU could maintain the inhibition for about 5 days whilst 50 mg/L and 10 mg/L of ATU could suppress the nitrification for only 2 days and less than 1 day respectively. In order to keep the ATU action for 10 days, 150 mg ATU/L had to be dosed to the system. In the case of low OHO concentration at 750 mg-COD/L ($\triangle$), the duration of ATU action was almost doubled because of the low biological ATU degradation rate.

Next, using the simulation plots, an empirical formula to obtain the duration of ATU action (day) from the initial ATU (mg/L) and initial OHO (mg-COD/L) was elaborated as shown in Equation (5). In the equation, the coefficients a_1, a_2 and b were intentionally selected to draw the curves slightly below the simulation plots, which also ensured obtaining the duration of ATU action in a conservative manner.

$$\begin{cases} \text{Duration of ATU action} = A \cdot \left(\text{Initial ATU}^b \right) \\ A = a_1 \times X_{OHO(0)} + a_2 \end{cases} \quad (5)$$

where, duration of ATU action (day), initial OHO concentration ($X_{OHO(0)}$, mg-COD/L), coefficients: $a_1 = -2.2 \cdot 10^{-5}$, $a_2 = 0.0535$, $b = 1.25$.

The equation was rewritten to obtain the permissive minimum ATU dose (mg/L) from $X_{OHO(0)}$ and desired duration of ATU action as shown in Equation (6). If OHO concentration in the activated sludge could be roughly estimated from the operating condition of the wastewater treatment plant and Activated Sludge Models (e.g. $X_{OHO(0)} = 40\%$ of the activated sludge COD), then the first-guess $X_{OHO(0)}$ could be used for the input parameter of the equation together with the desired duration of ATU action for the respirometric test. For convenience, the optimal ATU dose (safe minimum ATU dose) vs. the $X_{OHO(0)}$ is graphically shown in Figure 3(b). For instance, when the OHO concentration in the activated sludge sample was assumed to be about 600 mg-COD/L, the optimal ATU dose to suppress nitrification for 10 days was determined to be 82 mg ATU/L.

$$\text{Optimal ATU dose} = \left(a_1 \times X_{OHO(0)} + a_2 \right)^{-c}$$
$$\times \text{Desired duration of ATU action}^c$$
$$(6)$$

where, optimal initial ATU dose (mg/L), initial OHO concentration ($X_{OHO(0)}$, mg-COD/L), desired duration of ATU action (day), coefficients: $a_1 = -2.2 \cdot 10^{-5}$, $a_2 = 0.0535$, $c = 0.8$.

Impact of ATU dose on the analytical accuracy of heterotrophic OUR

Although the ATU inhibition threshold against AOO ($K_{I, \ AOO\_ATU} = 1.5$ mg-COD/L) was very low, the initial ATU
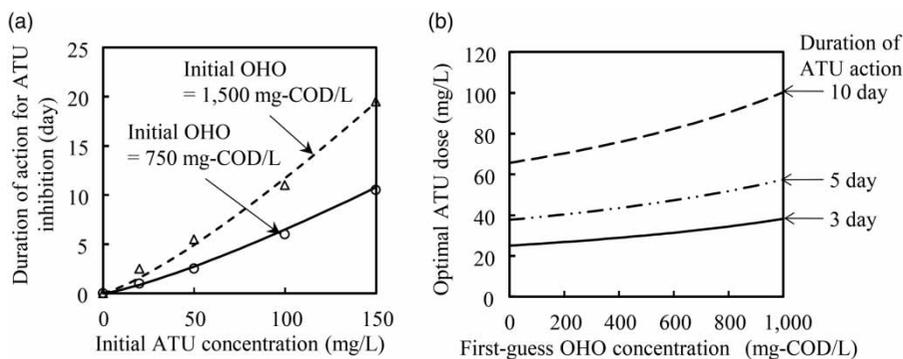

Figure 3 │ Optimal ATU dose for batch respirometric test (left: Estimated duration of ATU action against AOO activity (inhibition threshold = 0.5 mg-ATU/L, plots: simulation from the model (O: X_{OHO} = 1,500 mg-COD/L; $\triangle$: $X_{OHO(0)}$ = 750 mg-COD/L), lines: calculated from Equation (5)); right: optimal ATU dose calculated from initial OHO concentration to keep the duration of ATU action for 3 days, 5 days and 10 days).

dose should be carefully determined to keep the ATU concentration beyond the level throughout the respirometric test. On the other hand, too high an ATU dose and too high OHO should not be selected for the respirometric test because of additional heterotrophic oxygen uptake from the biological ATU oxidation; even the optimal ATU dose was determined from the above empirical equation. In the case that very high OHO was present in the activated sludge, correspondingly very high ATU had to be dosed. Addition of much ATU might result in noticeable overestimation of b_{OHO} unless OUR from ATU was properly subtracted from the monitored OUR.

As simulated in Figure 4(a), when the optimal ATU dose was determined to be 73 mg/L from Equation (6) to conduct an 8-day respirometric test with 750 mg-COD/L of $X_{OHO(0)}$, the simulated composite heterotrophic OUR ($OUR_{(t)}$) consisted of the biological ATU oxidation and the $OUR_{e(t)}$ gave about 0.25 d^{-1} of semi-logarithmic slope. As the slope was almost comparable to the true OUR_e ($b'_{OHO} = 0.24\ d^{-1}$), overestimation of b'_{OHO} from the the composite OUR was limited by only 4.1% ($=0.25/0.24-1$). On the other hand, the intersection of the Y-axis on the respirogram ($\equiv X_{OHO(0)}$) showed 133 mg-O_2/L/day of $OUR_{(0)}$ whilst the true $OUR_{e(0)}$ from the OHO decay was 94 mg-O_2/L/day. In this case, $X_{OHO(0)}$ was noticeably overestimated by 41.5% due to high ATU in the initial phase. Therefore addition of ATU should be further reduced in order to accurately estimate $X_{OHO(0)}$. Similarly, when the ATU dose was set to 150 mg/L for 1,500 mg-COD/L of $X_{OHO(0)}$, as shown in Figure 4(b), the OUR could not be linearly placed on the semi-logarithmic graph and wrongly gave high b'_{OHO} as well as unusually high $X_{OHO(0)}$. Considering these simulation results, dosing several 10 mg/L of ATU would be a practical permissive maximum for measuring b_{OHO}.

Friedrich (2016) also found that noticeably high OUR was yielded when very high ATU was dosed in the batch respirometric tests to monitor the endogenous respiration by OHO. The calculated b'_{OHO} of the activated sludge sample was overestimated to be almost twice as high as the true b'_{OHO}, in which biodegradation of ATU was supposed to take place. The semi-logarithmic slope of $OUR_{(t)}$ versus time in the initial phase was also unusually higher than those obtained in the latter phase, resulting in inconsistent b'_{OHO} over the experimental period. At the end of the experiment, an OUR peak was eventually created due to the recovery of nitrification. The simulations in this study could clearly demonstrate how the additional OUR and the inconsistent b'_{OHO} were built by the biological oxidation of ATU at high ATU concentration (Figure 4), as well as the production of the OUR peak after degradation of ATU (Figure 2(b) and 2(c)). To suppress the additional OUR derived from the high ATU concentration, intermittent addition of very low ATU to the respirometry vessel throughout the experiment might be an interesting option. In this way, $OUR_{(t)}$ could be controlled to approach $OUR_{e(t)}$.

To cope with the problem to estimate $X_{OHO(0)}$ under the presence of ATU and nitrifiers, three kinds of OUR simulations with lowered ATU dose were conducted as shown in Figure 5. When the ATU dose was set to only 5 mg/L under the presence of 25 mg N/L of NH_x (*a*: simulation marked with solid line −), the $OUR_{(0)}$ was almost identical to the true $OUR_{e(0)}$ (X_{OHO} only, *b*: simulation marked with dotted line …) until nitrification took place from day 1. Therefore, in order to estimate $X_{OHO(0)}$ from $OUR_{(0)}$ of activated sludge samples, the ATU dose should be reduced to this level. In such respirometric tests, since the initial ATU concentration was set to be as low as possible, suppression of nitrification during the initial
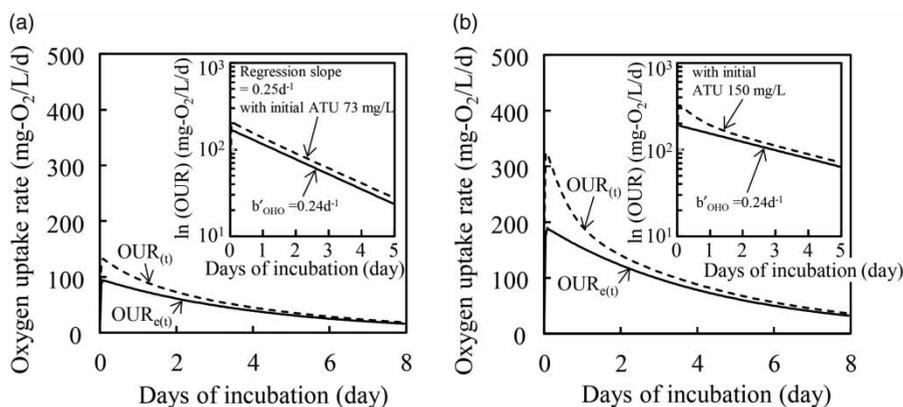

Figure 4 │ Simulated OHO oxygen uptake rate with and without ATU under absence of nitrifiers (left: $X_{OHO} = 750$ mg-COD/L, ATU = 73 mg/L; right: $X_{OHO} = 1,500$ mg-COD/L, ATU = 150 mg/L; line: endogenous respiration only ($OUR_{e(t)}$), dashed line: endogenous respiration + biodegradation of ATU ($OUR_{(t)}$)).

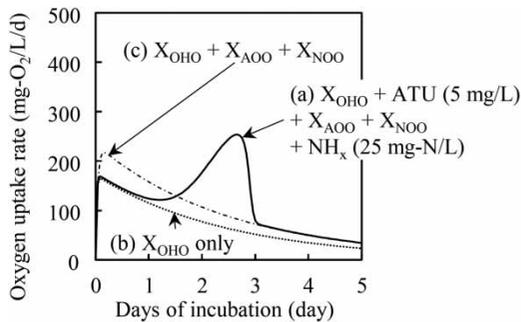Figure 5 | Simulated oxygen uptake rates with and without ATU, nitrifiers and ammonium-N (X_{OHO} = 750 mg-COD/L, X_{AOO} = 80 mg-COD/L, X_{NOO} = 40 mg-COD/L, NH_x = 25 mg-N/L; (a): X_{OHO} + ATU + X_{AOO} + X_{NOO} + NH_x; (b): X_{OHO} only; (c): X_{OHO} + X_{AOO} + X_{NOO}).

phase should be carefully checked. In fact, when no ATU was present in the system (also with no external addition of NH_x), the $OUR_{(t)}$ was always 40% higher than the true $OUR_{e(t)}$ of the endogenous respiration by OHO (*c*: simulation marked with dot-dashed line ---), resulting in overestimation of $X_{OHO(0)}$ by 40%. This was because the $OUR_{(t)}$ consisted of the true $OUR_{e(t)}$ and the cryptic oxygen uptake of nitrifiers, which was derived from the biodegradable-N of the decayed OHO; ($OUR_{(t)} \cong (1\text{-}f_U') \ b_{OHO} \cdot X_{OHO(t)} + 4.57 \cdot f_{XBN} \cdot (1\text{-}f_U') b_{OHO}' \cdot X_{OHO(t)} \cong 1.4(1\text{-}f_U')b_{OHO}' \cdot X_{OHO(t)} = 1.4 \cdot OUR_{e(t)}$). Considering that simultaneous measurements of ATU and/or nitrate during the respirometric test was cumbersome, intentional small addition of ammonium-N (*e.g.* 25 mg-N/L) and ATU (*e.g.* 5 mg/L) to the system was thought to be an alternative and attractive method which could visualise the suppression and recovery of nitrification. If an OUR peak was seen in the latter phase of the experiment, the $OUR_{(0)}$ could be judged to be created under the suppression of nitrification. In this case, the $OUR_{(0)}$ could be directly used to estimate $X_{OHO(0)}$. On the other hand, if no OUR peak was recognised, it could be deduced that the nitrifier present in the activated sludge sample would be negligibly small, and hence the b'_{OHO} could be simply calculated from the $OUR_{(t)}$ versus time.

CONCLUSION

Biological decomposition of allylthiourea was studied in order to evaluate the impact of the inhibitory concentration against nitrification on a respirometric test of activated sludge. From the study, the following results were obtained.

1. Allylthiourea was found to be biologically decomposed in the activated sludge, and nitrification took place within a

couple of days when high heterotrophs were present in the activated sludge sample.
2. Using a modified Activated Sludge Model No. 1 including two-step nitrification, the biological degradation of allylthiourea was kinetically modelled. From the kinetic model, empirical equations to estimate optimal ATU dose to determine the specific decay rate of heterotrophs were elaborated.
3. If heterotrophic biomass concentration in the activated sludge sample was limited to below 1,000 mg-COD/L, the oxygen uptake from the biological ATU oxidation at the optimal ATU dose seemed to be negligible in the calculation of the specific decay rate of heterotrophs. On the other hand, calculation of heterotrophic biomass concentration was found to be sensitive to the ATU dose. For its accurate estimation, experiments with low ATU concentration (e.g. 5 mg/L) are recommended.

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