Evaluating nitrite oxidizing organism survival under different nitrite concentrations

Bing Liu, Yifan Li, Jinzhu Wu, Yuanyuan Shao, Feiyong Chen, Jer-Horng Wu, Rajeev Goel IWA, Mitsuharu Terashima and Hidenari Yasui IWA

ABSTRACT

The objective of this study is to explore the optimal pre-treatment procedures and statistics methods for live/dead bacterial staining using nitrite oxidizing organism (NOO) as the research aim. This staining method was developed and widely utilized to evaluate activated bacterial survival situation, because it is direct and convenience to count live and dead bacteria amount by colour distinguishes (green/red) from pictures taken by microscope. The living cell (green colour) percentage and initial bacterial chemical oxygen demand (COD) could be used for accurate reaction rate calculation at the beginning of tests. While according to the physiological principles, the detection target was limited as the organism has a complete cell shape, that was applicable for the initial phase for decay stage (live cell → particulate dead cell), but it is impossible to evaluate the decayed soluble COD from particulate dead cell during whole reaction. To model the decay stage scientifically, a two-step decay model was developed to cater to the live/dead bacterial staining analysis of biological nitrite oxidizer under inhibition condition of high nitrite concentrations at 35 °C. As results of optimal pre-treatment, a three level ultrasonic wave with 45 seconds was explored, as a reasonable observed picture number, 30 sets with 95% confident interval for datasets statistics was summarized. A set of nitrite oxidizer inhibition test (total COD and oxygen uptake rates) under high nitrite concentrations was simulated using the above model and obtained experimental schemes. Additionally, the disintegration enhancement from particulate dead cell to soluble COD by nitrite was inspected and modelled on the basis of experimental datasets.

Key words | decay model, kinetics, live/dead staining, nitrite oxidizing organism (NOO), two-step

Bing Liu (corresponding author)
Yifan Li
Jinzhu Wu
Yuanyuan Shao
Feiyong Chen
Resources and Environment Innovation Institute,
School of Municipal and Environmental Engineering,
Shandong Jianzhu University,
Jinan, 250101,
China
E-mail: *b-liu@sdjzu.edu.cn*

Bing Liu
Mitsuharu Terashima
Hidenari Yasui IWA
Faculty of Environmental Engineering,
University of Kitakyushu,
1-1, Hibikino, Wakamatsu, Kitakyushu, 808-0135,
Japan

Jer-Horng Wu
Department of Environmental Engineering,
National Cheng Kung University,
No. 1 University Road, East District, Taiwan City 701,
Taiwan

Rajeev Goel IWA
Hydromantis Environmental Software Solutions Inc.,
Suite 1601, 1 James Street South, Hamilton, Ontario L8P4R5,
Canada

INTRODUCTION

Enumerate viable bacterial methods were focused and developed from several decades ago, due to the reasons of lack of specificity, incubation or preparation requirements, the universal application was not implemented (Boulos *et al.* 1999). A fluorescent stain composed of a mixture of two nucleic acid-binding stains (SYTO 9 and propidium iodide) was found (Boulos *et al.* 1999) and widely utilized on bacterial live/dead count by colour distinguished (green/red) from pictures taken by microscope (Hao *et al.* 2009; Karkashan *et al.* 2015; Hu *et al.* 2017; Liu *et al.* 2018; Nasrabadi *et al.* 2018; Wang *et al.* 2018; Yang *et al.* 2018). Presently, in most research using live/dead bacterial staining, the objective is to evaluate the live bacterial tendency for decay rate calculation, and activity transform explanation. In another way, this method could be used for the initial bacterial amount determination in biological wastewater treatment process simulation according to the viable bacterial percentage and initial biomass chemical oxygen demand (COD) concentration in a monotonous biological system, for instance, nitrite oxidizing bacteria.

Although the standard bacterial staining method was given by reagent maker, there are still many important

doi: 10.2166/wst.2020.138

effect factors during operation that were needed to standardize, as follows:

(1) Since the presence of cell clusters in the sludge sample hindered the accuracy of the cell counting in microscopy, a preliminary experiment to optimise dispersion was necessary.
(2) To calculate the average area for the whole population based on measured data and control the error to within a suitable range, picture number and confidence interval were selected properly.

According to the bacterial staining mechanism, the detection target was limited and the organism has a complete cell shape, that was applicable for the initial phase for decay stage (live cell → particulate dead cell) that could obtain the net growth/decay rate by viable bacterial amount (green area) (Hao *et al.* 2009; Liu *et al.* 2018), but it is impossible to evaluate the decayed soluble COD from particulate dead cell during the whole reaction. To model the decay stage scientifically, a two-step decay model (live cell → particulate dead cell → soluble COD) was needed to develop in actual activated sludge process simulation to cater common analysis issues (for instance, COD, oxygen uptake rates) and process performance.

The objective of this experimental study was to explore the optimal experimental pre-treatment conditions for enumerate viable bacterial methods using nitrite oxidiser as an example. Nitrite oxidiser is important in the N-removal process; its activity inhibition affects the achievement of partial nitritation (Liu *et al.* 2012, 2013), a sample number for statistics with suitable confidence interval. Based on reasonable datasets, a two-step decay model was developed and utilized in nitrite oxidation batch tests performed under different nitrite concentrations to verify the model sanity.

MATERIALS AND METHODS

NOB enrichment and bacterial staining

Nitrifying activated sludge was collected from a domestic wastewater treatment plant with membrane bioreactor (MBR) process having 100 days of SRT and intermittent aeration (Kitakyushu, Japan). The sludge was enriched in a 5-L sequential batch reactor (SBR) using synthetic wastewater containing $NaNO_2$ (500 mg-N/L), NH_4Cl (1.0 mg-N/L), KH_2PO_4 (0.13 mg-P/L) and Na_2PO_4 (1.67 mg-P/L). The pH, temperature and dissolved oxygen (DO) in the reactor were controlled at 7.3, 35 ± 0.5 °C and more than 5 mg/L, respectively. As the sludge was in dispersed form throughout the operation periods, the solid–liquid separation was performed using centrifugation. An SRT of 50 days was maintained in the SBR reactor. After 240 days of the operation, ammonia oxidising organism (AOO) in nitrifying activated sludge was washed out based on qPCR analysis, and the enriched SBR sludge (NOO) was used for the batch tests. Nitrite and nitrate concentrations were measured using ion chromatography (ICS-1000, Dionex, USA). Ammonium nitrogen and sludge COD were analysed based on *Standard Methods* (APHA 2012).

To distinguish living and dead bacteria from the sludge, LIVE/DEAD® BacLight™ bacterial viability kit (L-7012, Molecular Probes, USA) was used. The kit consisted of green fluorescent nucleic acid stain (SYTO® 9) and red-fluorescent nucleic acid stain (Propidium Iodide (PI)). In principle, the SYTO® 9 (green fluorochrome) could penetrate into cells from their intact cell membrane ('living cell') because of its small molecule size, whilst larger molecule PI (red fluorochrome) only penetrated damaged membrane ('dead cell'). When SYTO® 9 only was used, all bacterial cells were stained green. On the other hand, when both stains were used, PI penetrated into the 'dead' cell reduced the fluorescence from the SYTO® 9 resulting in cells labelled in red. In this way, the living cells (green) and the dead cells (red) could be individually counted (Hao *et al.* 2009).

The dispersed sludge samples (977 μL in each testing) were transferred into 1 mL plastic tubes together with 1.5 μL of SYTO® 9 and 1.5 μL of PI, and incubated in the dark for 15 min at room temperature. Then, glass slides with stained sludge samples (10 μL on each slide) were prepared to observe and photograph with a fluorescence microscope (Nikon ECLIPSE 80i, Japan; Nikon DS-Fi2, Japan) using fluorescence filters of GFP-B (excitation at 460–500 nm and emission at LP515 nm for green fluorescence) and CY3 (excitation at 545 ± 30 nm and emission at LP590 nm for red fluorescence), respectively (Lopez *et al.* 2005; Hao *et al.* 2009). The stained cell areas (μm^2) were visualised and measured with a binarized image analysing software (Quick Grain, Inotech Inc., Japan).

Pre-treatment for optimise bacterial dispersion

Since the presence of cell clusters in the sludge sample hindered the accuracy of the cell counting in microscopy, it is necessary to disperse the sludge by a pre-treatment process; on another hand, a long and/or high intensity pre-treatment might destroy the bacterial cell, thus, prior to the monitoring, a preliminary experiment to optimise the

degree of ultrasonication was conducted. For the preparation of staining experiments, 5 mL enriched SBR sludge (NOO) was taken from the flasks and centrifugally washed 3 times for 3 min at 10,000 rpm with 0.85% NaCl solution, then dispersed by ultrasonic wave (UD-200, TOMY, Japan) for 0, 15, 30, 45, 60 seconds at 6 kHz for optimal treatment time, and for 0, 2, 4, 6, 8, 10, 12, 14 kHz (from Level 0 to 7) at 30 seconds for optimal ultrasonic wave intensity selection.

Optimal data set number selection using statistics methods

To calculate the average area for all population based on measured data and control the error to within a suitable range, the average area for all populations was calculated by Equation (1) (Elise & Jonathan 2002), in which $\bar{x}$ is the average for all samples, xi is the value of sample No. i, U is sample standard deviation, and $F^1_{n-1}(\alpha)$ is the value under $(1-\alpha)$ confidence interval that can be obtained from appendix of statistics book. In this experiment, 5, 10, 20, 30, 40, 60 and 90 sets of samples under 99%, 97.5%, and 95% confidence interval were calculated for a proper sample number.

$$U = \sqrt{\frac{\sum (x_i - \bar{x})^2}{n-1}}$$
$$\bar{x} - U\sqrt{\frac{F^1_{n-1}(\alpha)}{n}} \le \mu \le \bar{x} + U\sqrt{\frac{F^1_{n-1}(\alpha)}{n}} \quad (1)$$

Respiration test and two-step decay model structure

The enriched NOO was taken and utilized for the respiration test, NOO incubation tests were in operation, the nitrite concentrations were kept at 0, 50, 500 and 2000 mg-N/L with high concentration nitrite (20,000 mg–N) supplied by syringe pump for 7 days at $35 \pm 0.2\,^{\circ}C$ in a temperature-controlled incubator whilst the DO in the incubation vessels was kept constant at over 6 mg-O_2/L. Oxygen uptake rate measurement (OUR), volatile suspended solids (VSS) were measured according to the standard methods (APHA 2005) and live/dead bacterial staining was operated by above described methods.

RESULTS

Pre-treatment for optimise bacterial dispersion

The comparison of before and after treatment by ultrasonication was shown in Figure 1. As shown in Figure 1, it appeared that 30–45 seconds of ultrasonication showed the highest ratio of living bacteria to the total visualised cells with reasonable confidence interval. When no ultrasonication or longer treatment was performed, both experiments showed lower ratios which were attributed to poor cluster dispersion and considerable cell disruption respectively.

In addition, when loading too much power input (more than level 3), the countable cells decreased accordingly, shown in Figure 2. Based on the results, 30 seconds ultrasonication at level 3 was applied to the pre-treatment.

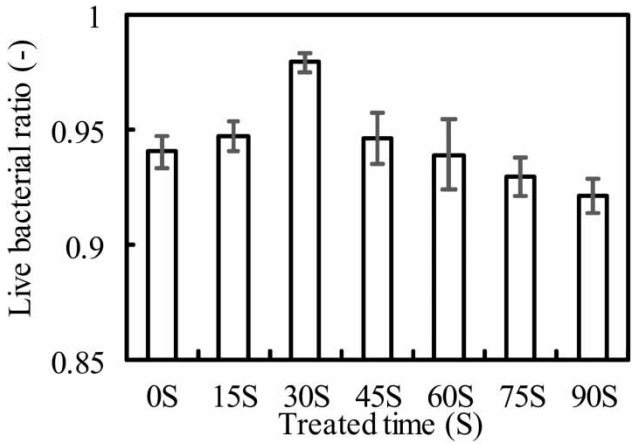

Figure 1 | NOO live bacterial ratio under different treatment time.

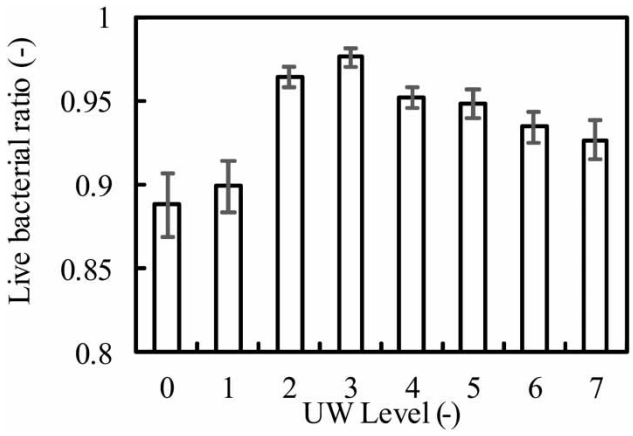

Figure 2 | NOO live bacterial ratio under UV levels.

In this experiment, 5, 10, 20, 30, 40 and 60 sets of samples under 99%, 97.5%, and 95% confidence interval were calculated for a proper sample number shown in Figure 3. Comparing all results in Figure 3, a conclusion could be obtained that using 30 photograph images per sludge sample, the areas were averaged, and its 95%-confidence interval was statistically determined finally due to acceptable error bar (below 3%) and sample number. As the dominant microorganism in the sample was supposed to be NOO, it was assumed that the visualised area corresponded to NOO biomass number.

Kinetics calculation for inhibition and disintegration

According to the optimal pre-treatment conditions, sample numbers and confidence intervals obtained from the above experiments, the living NOO and total NOO areas under different nitrite concentrations were observed and listed in Figures 4 and 5.

Based on previous research (Liu *et al.* 2018) and living NOO area slops in Figure 4, the Equation (2) was utilized to calculate $K_{I,NO2,poi}$ and power coefficient n. According to the total NOO area tendency in Figure 5, the total NOO amount general trend was similar with living NOO, one of reasons was predicted to affection from nitrite. When nitrite concentration was kept on a low level (for instance 50 mg-N/L in this study), NOO can grow smoothly since nitrite is the substrate for nitritation process. While nitrite beyond half-saturation concentration on poisoning ($K_{I,NO2,poi}$) with nitrite concentration increasing (500, 2000 mg-N/L in this study), the total NOO decrease tendency was enhanced, the experimental results could also explain the phenomenon nitrite enhanced waste activated sludge for methane fermentation (Wei *et al.*, 2017), the enhance disintegration by nitrite was summarized in Equation (3), the power coefficient m was attached to express the threshold type enhancement effect, which was similar with poisoning function. The parameter values $b_{NO2,poi}$, $K_{I,NO2,poi}$, n, $b_{NO2,l}$, $K_{i,NO2,l}$, m were calculated or simulated for slops (Liu *et al.* 2018) of living and total NOO based on Equations (2) and (3).

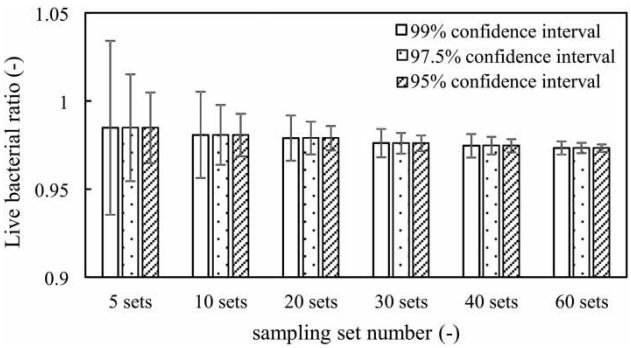

Figure 3 | Living bacterial ratio calculation under different sample numbers and confidence intervals.

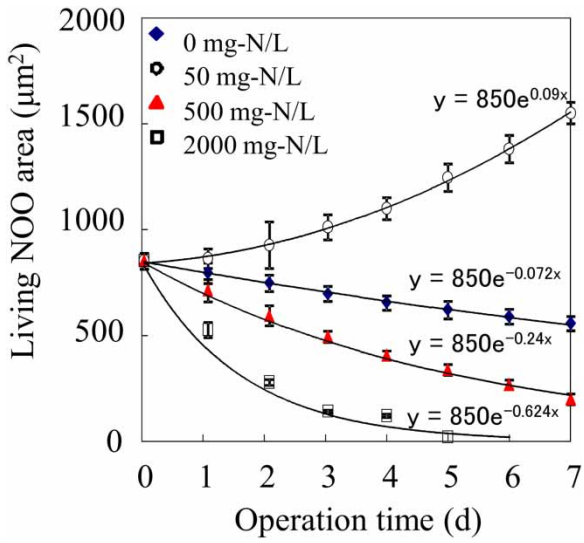

Figure 4 | Living NOO area calculation under different nitrite concentrations.

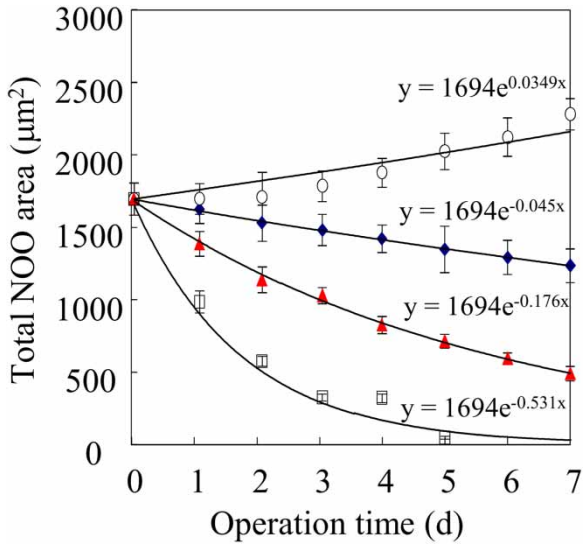

Figure 5 | Total NOO area calculation under different nitrite concentrations.

$$r = \mu - b_{tot}$$
$$\mu = \mu_{\max,\,NO2}\left(\frac{S_{NO2}}{K_{NO2}+S_{NO2}}\right)\left(\frac{K_{I,NO2,gro}}{K_{I,NO2,gro}+S_{NO2}}\right) \quad (2)$$
$$b_{tot} = b + b_{NO2,poi}\left(\frac{S_{NO2}^{n}}{K_{I,NO2,poi}^{n}+S_{NO2}^{n}}\right)$$

where r: net specific growth rate from nitrite (d^{-1}), μ: specific growth rate from nitrite (d^{-1}), b_{tot}: total specific decay rate (d^{-1}), $\mu_{max,NO2}$: maximum specific growth rate from nitrite (d^{-1}), S_{NO2}, nitrite concentration (mg-N/L), K_{NO2}: half-saturation coefficient of nitrite on growth (mg-N/L), $K_{I,NO2,gro}$: half-saturation coefficient of nitrite on growth inhibition (mg-N/L), b: inherent specific decay rate (d^{-1}), $b_{NO2,poi}$: specific nitrite poisoning rate (d^{-1}), $K_{I,NO2,poi}$: half-saturation coefficient of nitrite on poisoning (mg-N/L), n: power coefficient of nitrite poisoning (–).

$$b_{l,tot} = b_l + b_{NO2,l}\left(\frac{S_{NO2}^m}{K_{i,NO2,l}^m + S_{NO2}^m}\right) \quad (3)$$

where $b_{l,tot}$: total specific disintegration rate (d^{-1}), b_l: inherent specific disintegration rate (d^{-1}), $b_{NO2,l}$: specific nitrite disintegration rate (d^{-1}), $K_{i,NO2,l}$: half-saturation coefficient of nitrite on disintegration (mg-N/L), m: power coefficient of nitrite disintegration (–).

DISCUSSION

Two step decay model structure and simulation

According to the above observation results and calculation from living NOO and total NOO counting, based on IWA Activated Sludge Model No. 1 (ASM1) (Henze *et al.* 2000) a nitritation process in two-step nitrification containing enhanced decay ($X_{B,L} \rightarrow X_{B,D}$) and enhanced disintegration ($X_{B,D} \rightarrow S_B$) from nitrite was developed and modelled in Table 1.

In developed two-step decay model for biological nitrite oxidation process, based on ASM1, growth (m1), inherent decay (m2) and inherent disintegration (m4) was included. The nitrite inhibition on growth stage was described as non-competitive Monod-type function ($M_{NO2} \times I_{NO2}$) (Liu *et al.* 2018). To express the enhanced decay (m3) and disintegration (m5), the functions $I_{FNA,L}$ and $I_{FNA,D}$ in Table 1 were developed with power coefficient (n and m), which were free nitrous acid (FNA) concentration dependence switching functions.

Table 1 | Two step decay model for biological nitrite oxidation

m	Component → / Process ↓	S_{NO2}	S_{NO3}	S_{O2}	$X_{B,L}$	$X_{B,D}$	$S_{B,}$	X_U	Rate (mg-COD/L/d)
1	Growth	$-\frac{1}{Y}$	$+\frac{1}{Y}$	$-\frac{1.14-Y}{Y}$	+1				$\mu \times M_{NO2} \times I_{NO2} \times X_{B,L}$
2	Inherent decay ($X_{B,L} \rightarrow X_{B,D}$)				−1	+1			$b_D \times X_{B,L}$
3	Enhancement decay by nitrite ($X_{B,L} \rightarrow X_{B,D}$)				−1	+1			$b_{D,FNA} \times I_{FNA,L} \times X_{B,L}$
4	Inherent disintegration ($X_{B,D} \rightarrow S_B$)					−1	*1-f_U*	f_U	$k_D \times X_{B,D}$
5	Enhancement disintegration by nitrite ($X_{B,D} \rightarrow S_B$)					−1	*1-f_U*	f_U	$k_{D,FNA} \times I_{FNA,D} \times X_{B,D}$
		Nitrite mg-N/L	Nitrate mg-N/L	Oxygen mg-O_2/L	Active nitrite oxidising bacteria mg-COD/L	Dead nitrite oxidising bacteria mg-COD/L	Soluble COD from dead cell mg-COD/L	Particulate inert mg-COD/L	$I_{NO2} = \frac{K_{I-NO2}}{K_{I-NO2} + S_{NO2}}$
									$I_{FNA,L} = \frac{S_{FNA}^n}{S_{FNA}^n + K_{FNA,L}^n}$
									$I_{FNA,D} = \frac{S_{FNA}^m}{S_{FNA}^m + K_{FNA,D}^m}$

Model validation

Since the enriched NOO was cultivated in open jar fermenters, besides NOO, a small amount of ammonia oxidation organism (AOO), ordinary heterotrophic organism (OHO) and unbiodegradable organic product from cell decay ($X_{U,Org}$) were speculated in an experimental system (Liu *et al.* 2018). Although AOO ad OHO were possible to be inactivated by FNA, considering the amount, the effect was neglected for model simplification.

In simulation for OUR and VSS, the two-step decay model for biological nitrite oxidation in Table 1 and related model of AOO, OHO, hydrolysis and ammonification process in ASM1 were utilized, except the parameters in Table 1, the other parameters values were determined as the same procedures as the previous research (Liu *et al.* 2018). A process simulator (GPS-X, Hydromantis Environmental Software Solutions, Inc., Canada) (Kappeler & Gujer 1992; Henze *et al.* 2000) were used for reproduced the experimental plots of OURs (round plots) and VSS (triangle plots) shown in Figure 6. The parameters values were listed in Table 2.

CONCLUSIONS

Nitrite oxidizing organism survival under different nitrite concentrations was explored and evaluated; the following results were obtained in this study.

- The poisoning phenomena were visualised using live/dead staining, Live/dead staining experimental conditions and corresponding statistics method were explored and summarized.
- The disintegration of the decayed biomass was accelerated under high nitrite concentration and modelled applying FNA-dependence thresholds function on the newly defined disintegration processes. The OUR and VSS were simulated successfully using constructed functions and models.

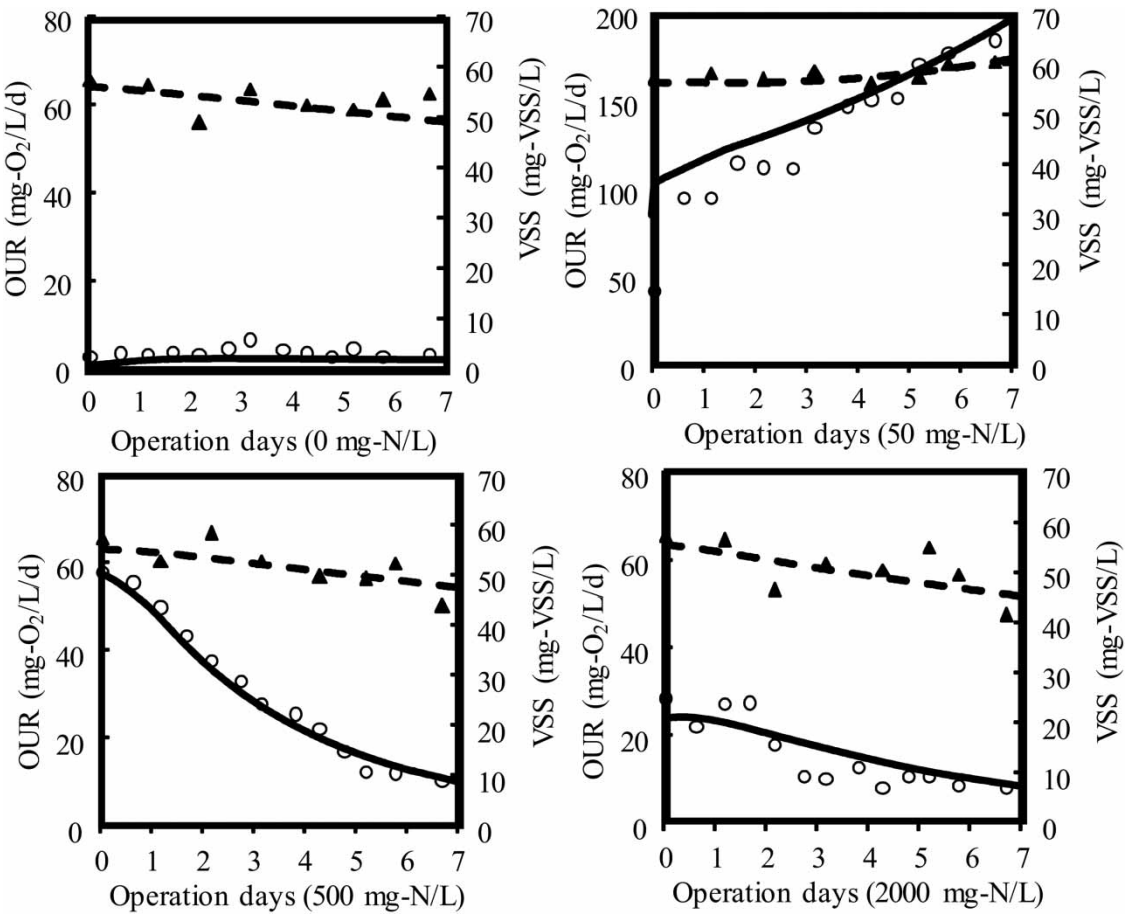

Figure 6 | OUR and VSS simulation results under different nitrite concentrations (OUR plots symbol: ○; VSS plots symbol: ▲).

Table 2 | Kinetic and stoichiometric parameters for biological nitrite oxidation

Symbol	Value	Item	Unit
NOO parameters			
Y	0.03	Yield of NOO	g-COD/g-N-nitrite (Ostace *et al.* 2011)
f_U	0.20	Production of particulate inert	g-COD/g-COD (Henze *et al.* 2000)
μ_{max}	0.26	Maximum specific growth rate	d^{-1} (at 35 °C)
K_{NO2}	35.0	Half-saturation coefficient on growth	mg-N-nitrite/L
b	0.072	Specific ordinary decay rate	d^{-1} (at 35 °C)
$b_{NO2,l}$	0.0048	Specific disintegration rate	d^{-1} (at 35 °C)
$b_{NO2,poi}$	0.552	Specific maximum poisoning rate by FNA	d^{-1} (at 35 °C)
$K_{I,NO2,poi}$	0.317	Half saturation coefficient on poisoning by FNA	mg-N-FNA/L
n	10.0	Power coefficient on poisoning by FNA	–
$b_{NO2,l}$	0.528	Specific maximum disintegration rate by FNA	d^{-1} (at 35 °C)
$K_{NO2,l}$	0.317	Half saturation coefficient on disintegration by FNA	mg-N-FNA/L
m	10.0	Power coefficient on disintegration by FNA	–
AOO parameters			
Y	0.208	Yield of AOO	g-COD/g-N-nitrite (Henze *et al.* 2000)
f_U	0.08	Production of particulate inert	g-COD/g-COD (Henze *et al.* 2000)
μ_{max}	6.0	Maximum specific growth rate	d^{-1} (at 35 °C) (Henze *et al.* 2000)
K_S	6.0	Half-saturation coefficient on growth	mg-N-nitrite/L (Henze *et al.* 2000)
b_{AOO}	0.04	Specific ordinary decay rate	d^{-1} (at 35 °C) (Henze *et al.* 2000)
$b_{AOO,l}$	0.04	Specific disintegration rate	d^{-1} (at 35 °C) (Henze *et al.* 2000)
OHO parameters			
Y	0.666	Yield of OHO	g-COD/g-N-nitrite (Henze *et al.* 2000)
f_U	0.08	Production of particulate inert	g-COD/g-COD (Henze *et al.* 2000)
μ_{max}	6.0	Maximum specific growth rate	d^{-1} (at 35 °C) (Henze *et al.* 2000)
K_S	0.5	Half-saturation coefficient on growth	mg-N-nitrate/L (Henze *et al.* 2000)
b_{OHO}	0.062	Specific ordinary decay rate	d^{-1} (at 35 °C) (Henze *et al.* 2000)
$b_{OHO,l}$	0.062	Specific disintegration rate	d^{-1} (at 35 °C) (Henze *et al.* 2000)
Hydrodisintegration process			
r_{Hyd}	3.0	Maximum specific hydrodisintegration rate	d^{-1} (at 35 °C) (Henze *et al.* 2000)
K_{Hyd}	0.03	Slowly biodegradable substrate half saturation coefficient	g-COD/g-COD (Henze *et al.* 2000)
Ammonification process			
r_{AMM}	0.08	Ammonification rate	m^3/g-COD/d (Henze *et al.* 2000)

- The model presented in this study was to express the loss of active NOO biomass due to poisoning and FNA-dependence enhanced disintegration, which was a distinct interpretation against the conventional models using competitive/non-competitive inhibition on growth stage. Therefore, the study could be a critical platform to improve the understanding of microbial inhibition and activated recovery phenomena.

ACKNOWLEDGEMENT

This work was supported by Science Foundation of Shandong Jianzhu University (Grant No. XNBS1824), Shandong Key Research and Development Program (No. 2019GSF109064, No. 2019GSF109088), National Natural Science Foundation of China (Youth Fund) (No. 41907204), National students' platform for innovation and entrepreneurship training program (201910430002), Japan

Society for the Promotion of Science (Grants in aid for scientific research, No. 22404003, 23658278 and 22254004) and talent incubation plan of universities youth innovation team of Shandong Province (innovation research team of water environment green remediation technology). The authors would like to thank Jer-Horng Wu, National Cheng Kung University, Taiwan for his help on identifying microbial species in the nitrifying sludge.

REFERENCES

APHA 2005 *Standard Methods for the Examination of Water and Wastewater*, 21st edn. American Publishers Health Association, Washington, DC, USA.

APHA, AWWA & WEF 2012 *Standard Methods for the Examination of Water and Wastewater*. American Public Health Association, American Water Works Association, Water Environment Federation, Washington, DC, USA.

Boulos, L., Prévost, M., Barbeau, B., Coallier, J. & Desjardins, R. 1999 LIVE/DEAD BacLight: application of a new rapid staining method for direct enumeration of viable and total bacteria in drinking water. *Journal of Microbiological Methods* **37**, 77–86.

Whitley, E. & Ball, J. 2002 Statistics review 2: samples and populations. *Critical Care* **6** (2), 143–148.

Hao, X., Wang, Q., Zhang, X., Cao, Y. & van Loosdrecht, M. C. M. 2009 Experimental evaluation of decrease in bacterial activity due to cell death and activity decay in activated sludge. *Water Research* **43**, 3604–3612.

Henze, M., Gujer, W., Mino, T. & van Loosdrecht, M. C. M. 2000 Activated sludge models ASM1, ASM2, ASM2D, ASM3. IWA Scientific and Technical report No. 9. IWA Publishing, London, UK.

Hu, W., Murata, K. & Zhang, D. 2017 Applicability of LIVE/DEAD BacLight stain with glutaraldehyde fixation for the measurement of bacterial abundance and viability in rainwater. *Journal of Environmental Science* **51**, 202–213.

Kappeler, J. & Gujer, W. 1992 Estimation of kinetics parameters of heterotorophic biomass under aerobic conditions and characterization of wastewater for activated sludge modelling. *Water Science and Technology* **25** (6), 125–139.

Karkashan, A., Khallaf, B., Morris, J., Thurbon, N., Rouch, D., Smith, S. R. & Deighton, M. 2015 Comparison of methodologies for enumerating and detecting the viability of *Ascaris* eggs in sewage sludge by standard incubation-microscopy, the BacLight Live/Dead viability assay and other vital dyes. *Water Research* **68**, 533–544.

Liu, B., Daisuke, N., Ian, J., Rajeev, G. & Hidenari, Y. 2012 A kinetic expression for the growth and decay of nitrite oxidising bacteria. *Japanese Journal of Water Treatment Biology* **48** (3), 89–97.

Liu, B., Ian, J., Daisuke, N., Rajeev, G. & Hidenari, Y. 2013 A benchmark simulation to verify an inhibition model on decay stage for nitrification. *Water Science and Technology* **68** (6), 1242–1250.

Liu, B., Terashima, M., Quan Nguyen, T., Ha Nguyen, T., Chieu, L. V., Goel, R. & Yasui, H. 2018 High nitrite concentration accelerates nitrite oxidising organism's death. *Water Science and Technology* **77** (12), 2812–2822.

Lopez, C., Pons, M. N. & Morgenroth, E. 2005 Evaluation of microscopic technology (epifluorescence microscopy, CLSM. *TPE-LSM*) as a basis for the quantitative image anadisintegration of activated sludge. *Water Research* **40** (8), 1519–1530.

Nasrabadi, A. M., An, S., Kwon, S.-B. & Hwang, J. 2018 Investigation of live and dead status of airborne bacteria using UVAPS with LIVE/DEAD® BacLight Kit. *Journal of Aerosol Science* **115**, 181–189.

Ostace, G. S., Cristea, V. M. & Agachi, P. S. 2011 Cost reduction of the wastewater treatment plant operation by MPC based on modified ASM1 with two-step nitrification/denitrification model. *Computers and Chemical Engineering* **35**, 2469–2479.

Wang, Q., Song, K., Hao, X., Wei, J., Pijuan, M., van Loosdrecht, M. C. M. & Zhao, H. 2018 Evaluating death and activity decay of Anammox bacteria during anaerobic and aerobic starvation. *Chemosphere* **201**, 25–31.

Wei, W., Qilin, W., Liguo, Z., Andrew, L., Haoran, D., Damien, J. B. & Zhiguo, Y. 2017 Free nitrous acid pre-treatment of waste activated sludge enhances volatile solids destruction and improves sludge dewaterability in continuous anaerobic digestion. *Water Research* **130**, 13–19.

Yang, Y., Li, C. & Hou, L.-a. 2018 Impact of dead cells on biofouling and pharmaceutically active compounds retention by NF/RO membranes. *Chemical Engineering Journal* **337**, 51–59.

First received 6 December 2019; accepted in revised form 18 March 2020. Available online 27 March 2020