Critical analysis of decay rate measurement methodology

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Abstract. In modeling studies, estimation of microorganisms kinetic parameters set is a key element for proper model operation and predictability. Nitrification process is very often, a crucial element of the wastewater treatment systems as bacteria responsible for ammonium and nitrite oxidation are slow growing microorganisms, making whole nitrification process vulnerable to external factors i.e. temperature, inhibition and load fluctuations. Growth and decay rate of nitrifiers decide about amount nitrifying biomass in the wastewater treatment plants, thus the nitrification efficiency. Paper presents analysis of the decay rate (b_a) estimation methodology based on respirometric assays measuring the oxygen uptake rate (OUR). Evaluation of this simple and cheap method was made based on decay estimation tests performed on sludge samples from side-stream partial nitritation reactor treating reject water from digested sludge dewatering. Database obtained from these tests were analyzed to evaluate the impact of respirometric assay duration on calculated decay rate values. 11 time ranges were selected for the performed analysis. Calculated b_a values were compared showing the optimal test duration between 5–6 hours, while test shorter than 2 hours resulted in unsatisfactory b_a outcome.

1 Introduction

1.1 The idea of decay rate

In activated sludge models (ASMs), decay rate (b_a, d^{-1}) is a part of the function describing net microbial growth rate presented in equation 1 [1]. In case of autotrophic, nitrifying biomass, a simplified version (omitting inhibition and operational conditions) of this function describes the relationship between the biomass yield coefficient (Y_A, g COD/g NH_4-N), decay rate, solids residence time (SRT, d) and the oxidized ammonium load ($NH_{4,ox}$, g NH_4-N/d) and relates those parameters to amount of biomass produced daily:

$$X_A = Y_A \cdot \frac{1}{1+b_A \cdot SRT} \cdot NH_{4,OX}, \text{[g COD/d]} \quad (1)$$

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where:
Y_A – nitrifiers yield coefficient, g COD/g NH_4-N
b_A – nitrifiers decay rate, d^{-1}
SRT – solids residence time, d
$NH_{4,OX}$ – oxidized ammonium load, g NH_4-N/d

Early versions of ASM models assumed the decay rate as integrated coefficient representing many different processes. Recent versions of these models (ASM3) was developed including number of components as presented in Figure 1. Main process included in this coefficient represents cell death in time due to natural life cycle. Other processes included in this parameter are: endogenous respiration, cell lysis, motility and predation due to protozoa activity [2].

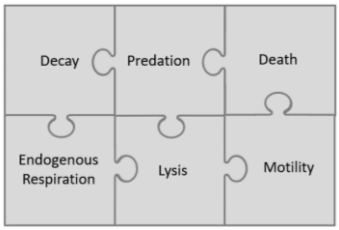

Fig. 1. Processes included in decay processes in oxic conditions.

Determination of the decay rate coefficient requires proper modification in the activated sludge operational regime. To measure the rate of nitrifying biomass loss, its growth must be minimalized. Due to these requirements, during the decay rate estimation test, activated sludge sample is kept in starvation conditions without substrate addition. The only source of substrate available for the bacterial growth are products obtained due to biomass decay processes. Next, the change of process kinetics under starvation conditions is investigated in specified time intervals to evaluate the nitrifiers loss.

Duration of the starvation period may vary depending on reports and was in range from 1 to 30 days [3, 4]. Characteristics of the decay rate coefficient, which is related to daily change, induces required intervals between successive process rate tests at 24 hours. Literature reports considering decay rate in aerobic conditions indicates that nitrifiers mass drops to 20% and 17% of the initial mass after 10 and 17 days of starvation, respectively [4, 5].

Based on available reports, analysis of published data about nitrifiers decay rate tests in aerobic conditions was performed and summarized in Table 1. In each of reviewed studies, decay rate was determined for both groups of nitrifiers or separately for each group of nitrifiers separately (ammonia oxidizers, AOB or nitrite oxidizers, NOB) if such data was available. Except operational conditions of each test (pH and temperature) also evaluation method of process kinetics and starvation period length was highlighted.

The lowest decay rate was significantly different from other reports and was equal 0.02 d^{-1} [6]. However some explanations can be found in the methodology of that test which was performed on pure cultures of nitrifiers feed with synthetic wastewater. Such conditions are significantly different from observed in activated sludge thus observed decay rate can be lower due to no protozoa grazing or neglected impact of toxic substances causing bacterial death or enhancing cell lysis. Maximal coefficient rate was 0.43 d^{-1} observed in *Nitrosomonas europea* (AOB) also cultivated in laboratory conditions [7]. Range of decay rate coefficient observed in conditions similar to full-scale activated sludge systems (mixed-cultures) was between 0.13 and 0.4 d^{-1}. Selected method of measuring

biomass activity probably also had impact on the final decay rate result, however in reports where oxygen uptake rate was used the spread of the results was smaller.

Table 1. Values of decay rate in the literature (measurement method: OUR – respirometric assay, NB – nitrification rate test, Model – mathematical model calibration). Test performed on pure cultures signed with (*).

AOB	(AOB+NOB)	NOB	pH	Temp	Method	Starv. period	Literature
d^{-1}	d^{-1}	d^{-1}	-	°C	-	d	
	0.19 ± 0.05		7.3–7.6	12	OUR	7 day	Siegrist et al. (1999)
	0.21 ± 0.05		7.3–7.6	20	OUR	7 day	Siegrist et al. (1999)
	0.153 ± 0.02		7.7 ± 0.2	20	NB	9 day	Lee and Oleszkiewicz (2003)
	0.43		7.5–7.8	28	OUR		Nowak et al. (1994)
	0.40			30			Slazer (1992)
0.43*			7.4	30	OUR	14 day	Leenen et al. (2000)
	0.05			10	Model		Henze et al. (2000)
0.02 ± 0.002*		0.08 ± 0.000*	7.5	20	NB	30 day	Salem et al. (2005)
0.20 ± 0.016		0.21 ± 0.024	7.5	20	NB	30 day	Salem et al. (2005)
0.35			7.3	20	NB	14 day	Oleszkiewicz (2011a)
0.15			7.5	20	Model	9 day	Siegrist et al. (2006)
	0.175 ± 0.002		7.0–8.0	20	NB	6 day	Zhou et al. (2015)
	0.13			10	NB		Choubert (2008)
0.28		0.27	7.3	20	NB	3 day	Oleszkiewicz (2011b)
	0.19		7.2	20	NB	6 day	Dold (2005)
	0.06		7.3	20	NB	5 day	Katehis (2002)
	0.218			25	NB	1 day	Lesouef (1992)
	0.13			20	Model	1 day	Lesouef (1992)
	0.24			20	OUR	30 day	Julián Carrera (2011)
	0.08–0.36		7.6–7.8	30	OUR	5 day	Martinage (2000)
0.26		0.07			NB		Volcke E.I.P. (2006)
0.24 ± 0.02		0.35±0.02	5.6	29	NB	7 day	Wenlong Liu (2017)

No detailed information is provided about taking into account the protozoa activity influencing the overall observed oxygen uptake rate and additionally removing nitrifiers from the system. The same mechanism can be related to heterotrophic processes consuming oxygen, thus affecting observed OUR. These phenomena should be considered during calculations of decay coefficient by use of correction factors including influence of estimated non-nitrifying biomass activity. As the internal substrate source production is strictly connected with the processes responsible for cell destruction, its rate is relatively stable throughout whole test. To include this phenomenon in the final results, rate of endogenous respiration of sludge sample should be determined in each test and subtracted from the measured OUR. Considering other parameters, like pH and temperature, no significant differences between presented studies was noticed. In most of presented reports selected temperature and pH were 20°C and about 7.5, respectively.

1.2 Decay rate methodology

There is a number of methods to determine the decay rate for a activated sludge. Regardless of the used method, decay rate is determined by a comparative analysis in a time period. Most of the laboratory experiments (Table 1) determine this coefficient based on the isolation of the activated sludge sample from the substrates necessary for the multiplication of individual groups of bacteria in certain process conditions: aerobic, anoxic or anaerobic.

The most common methods in this regard are primarily measurements of the process kinetics, such as OUR or substrate utilization rate by microorganisms (i.e. Ammonia Uptake Rate, AUR). In the case of autotrophic organisms such as nitrifiers, it will be ammonium nitrogen for AOB or nitrite nitrogen for NOB. In the case of heterotrophic organisms, the monitored substrate will be organic carbon. The nitrification rate test is based on the determination of the dynamics of decreasing or increasing in individual nitrogen forms during the experiment. Based on the dynamics of changes, the maximal bacterial activity is determined in optimal conditions, without substrate limitation and potential inhibition by too high substrate concentration [8–10].

The OUR test is based on oxygen concentration depletion measurements due to bacterial respiration. Oxygen is, alongside ammonium and nitrite, the main substrate for the metabolic processes of nitrifiers. To observe the maximal dynamics of process, and thus the oxygen consumption, measurements have to be performed in a significantly higher range of dissolved oxygen concentration than the nitrifiers oxygen affinity, which in ASM models is set default at 0.5 g O_2/m^3 [1]. At the moment of aeration there is a situation where the half-saturation constants is much lower than the substrate actual oxygen concentration, hence the mathematical description of the reaction can be represented by the zero order equation, where the reaction rate is directly determined based on the decrease in oxygen concentration over time. In addition, it should be ensured that the system does not provide organic carbon, which would result in measuring the activity of several groups of bacteria, including heterotrophs.

Except the experimental methods for determining the decay rate highlighted previously, there are also molecular analysis techniques based on the recognition of RNA or DNA of specific microorganisms, by the selective determination of genes or enzymes. These techniques allows to determine the amount of bacteria in sample. In this group of methods, the main ones are those based on fluorescence, where the reaction to the introduction of specific antibodies give a visual response. The most commonly used molecular analysis techniques are the FISH (fluorescent in situ hybridization) method based on visual fluorescence identification and the PCR (polymerase chain reaction) method, which uses the amplification of available genetic material by introducing an enzyme catalysing the synthesis of cell genetic information carriers (DNA/RNA) [11]. It should be emphasized that the molecular methods, at the current state of knowledge, are not significantly developed and are economically disadvantageous due to the cost of conducting such analyses.

Another group of methods for determining the decay rate is the use of existing mathematical models. Based on the principle of mass conservation, mathematical models describing transformation processes and removal of biogenic compounds have been created. The first advanced model was published in 1985 under the name ASM1. It gained general approval and over the years it was developed with additional elements (ASM2, ASM2d, ASM3). In subsequent versions, there is a distinction and separation of processes carried out by individual groups of bacteria. In the ASM2, ASM2d and ASM3 models, the default value for decay rate for nitrifiers is $b_a = 0.15\ d^{-1}$ [1]. The effectiveness of the model depends on the input values. Determination of the constant of decay rate with this method is usually based on prior calibration of the model, which will allow a meaningful reference of the simulation to the actual conditions, which also requires the use of laboratory methods.

This study focused on measuring OUR in aerobic conditions for nitrifiers. OUR tests are characterized by the relatively lowest costs of all the aforementioned methods. In addition, it does not require highly specialized equipment contrary to the molecular techniques. Also, the OUR method significantly minimizes the use of chemical reagents, limiting them to the dosing of the substrate. The test itself is relatively simple, both

regarding the methodology and the subsequent analysis of the collected data. Based on these premises, respirometric method was used during the described experiment.

1.3 Decay rate in side-stream treatment

Contrary to mainstream nitrogen removal systems, there are only few reports about determination of the decay rate of nitrifiers present in side-stream systems treating high-strength ammonium wastewater (i.e. landfill leachate or digester supernatant) [12, 13]. Available data about decay coefficient in such systems is gained due to model calibration rather than experimental determination. However, observed decay rates might be higher than in the conventional nitrification systems due to higher temperature, especially considering membrane bioreactors with long SRT [14]. Surprisingly, in side-stream attached biomass systems, decay rate is presented as equal or even lower than in conventional systems, both for AOB and NOB [15]. The performed literature analysis revealed no attempt for experimental determination of nitrifiers decay rate coefficient in side-stream systems so far.

2 Materials and methods

2.1 Starvation Reactor parameters

A CSTR reactor (150dm^3) was used as the starvation reactor. Reactor was equipped with a mechanical mixer, aeration system controlled by on-line dissolved oxygen (DO) probe (Hach LDO101) to continuously maintain DO level between 5–7 g O_2/m^3. Temperature was kept above 25°C using a single heating segment, thus while ambient temperature was higher than this value, also the reactor content temperature was increasing. During reactor operation alkalinity was kept at non-limiting level, above 20 mval/dm^3.

2.2 Inoculum

Biomass used in this study was an activated sludge from a partial nitritation (PN) SBR treating reject water from digested sludge dewatering to obtain NO_2/NH_4 ratio suitable for an Anammox reactor. PN reactor was operated under following conditions: SRT ~3 days, temperature 25°C, low DO level (~1 g O_2/m^3) and pH 6.4–6.6. Ammonium and nitrite concentration level in this reactor varied between 300–350 mg NH_4-N/dm^3 and 350–400 NO_2-N/dm^3, respectively.

2.3 OUR test methodology

Two decay rate tests (Series 1 & Series 2) were performed using sludge from PN reactor. Sludge samples from the starvation reactors were transferred to a lab-scale reactors (4 dm^3) with mixing, aeration, temperature and pH control to measure OUR in following days. 5 respirometric assays in 5 days were completed during Series 1, while Series 2 took 6 days with 5 respirometric assays. OUR tests were performed daily in 26°C and 30°C for Series 1 and Series 2, respectively.

Each respirometric assay started from the initial substrate concentration (50 mg NH_4-N/dm^3 added as NH_4Cl) and alkalinity as $NaHCO_3$. pH was controlled at 7.5 and monitored on-line by a pH-electrode (Hach, PHC101). To determine OUR, oxygen oscillated between 4–7 g O_2/m^3. Providing such conditions, nitrification process was not

limited or inhibited by any factors and allowed to determine the nitrifiers mass loss in following days.

2.5 Data analysis

The values of the OUR's recorded in subsequent days were lower and on this basis the value of decay rate was determined. The analysis of each batch test allowed to determine the reliable instantaneous values of the oxygen uptake of the process. For the Series 1 a total of 160 instantaneous OUR values were determined, and 450 in the Series 2. Data analysis was carried out in two consecutive stages. The first stage was based on the analysis of established time ranges in terms of instantaneous OURs. Ranges were determined on the basis of the dynamics of instantaneous rate variation and are presented in Table 2 (ranges 1–9). Further for individual ranges, the analysis of cumulative oxygen consumption from the beginning of the experiment to the moment determined by the range was performed. The last step (stage 2) was the elimination of a specific time interval (1 h and 2 h from the beginning of the test), thus creating successive time intervals (ranges 10 and 11) based on the ranges selected from stage 1.

Table 2. Accepted time ranges for stage 1 and stage 2.

	Series I	**Series II**
Stage I		
Range 1	0.5 h	0.5 h
Range 2	1 h	1 h
Range 3	2 h	2 h
Range 4	3 h	3 h
Range 5	5 h	5 h
Range 6	-	7 h
Range 7	-	9 h
Range 8	-	11 h
Range 9 (all)	All (6.6 h)	All (12.8 h)
Stage II		
Range 10 (cut time 1 h)	5.6 h	8 h
Range 11 (cut time 2 h)	4.6 h	7 h

The range 9 was determined to include all 86 and 180 OUR values for Series 1 and Series 2, respectively. For each time range, the total oxygen consumption was determined at that time and the value decreased relative to the first test. Due to the use of the longest range for the first series of measurements, it was decided to extend the second measurement series to about 12 hours in order to search for a period for which time extension will not cause a significant change in observed values. Time of approx. 6–12 h is too short and potential effect of autotrophic biomass growth will not affect the observed OUR values. The obtained results were subjected to the least-squares estimation using the non-linear Gauss-Newton regression method.

3 Results

The first stage of data analysis mentioned in section 2.2 was to determine OUR's for selected ranges depending in each series. The results of the measured values are presented in the diagrams below (figure 2 and 3).

Measured values clearly shows the decrease of the observed OUR value in following days, obviously due to nitrifiers decay process. During the initial 2 hours of each respirometric assay, very low values of OUR were noticed probably due to a bacterial lag phase after starvation period. This phenomenon led to the second stage of performed analysis where first one or two hours were excluded from the database (range 10 and 11, respectively).

Cumulative values for each day was compared to first day and percentage values was determined. Result are summarized in Table 3.

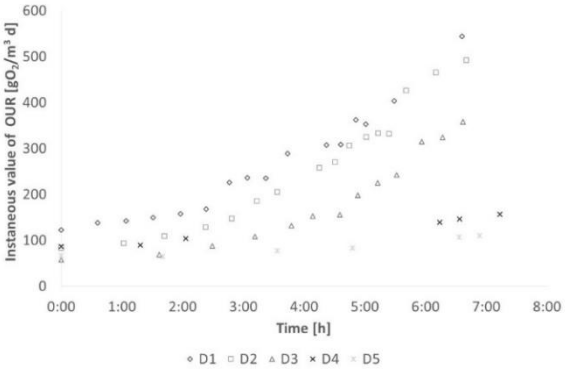

Fig. 2. OUR's during Series 1 in days 1–5.

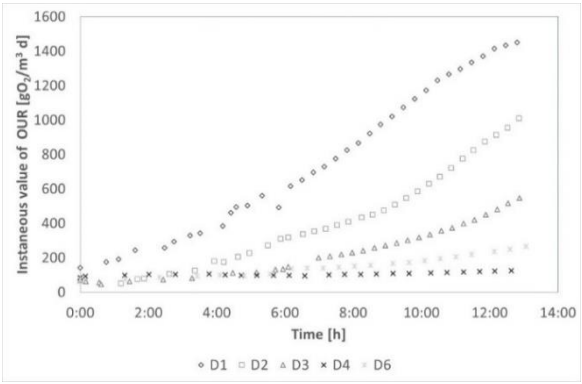

Fig. 3. OUR's for series no. 2 from day 1 to day 6.

The collected data was used to calculate the decay rate by fitting the experimental data to model. Calculated decay coefficient was corrected due to temperature as proposed in previous studies using equation 2 [11, 16]. Empirical coefficient of for (θ) temperature changes was 1.072. The b_a^t calculated for Series 1 & 2 (26°C and 30°C, respectively) and values corrected to 20°C are presented in Table 4.

For Series I the best model fit was for range 9 (6.6 h – full time of experiment) while for Series 2 there was no significant difference in curve fitting for ranges from 7 to 9. Estimation results was show in fig. 4.

Table 3. Values of cumulative oxygen consumption (Stage 1 of the analysis) compared to day 1.

	Day 2	Day 3	Day 4	Day 5	Day 6
Series I					
Range 1	66.6%	46.0%	67.2%	51.2%	-
Range 2	66.0%	45.5%	65.1%	49.0%	-
Range 3	68.1%	45.7%	63.8%	46.1%	-
Range 4	69.2%	45.9%	60.6%	42.2%	-
Range 5	76.7%	47.8%	50.1%	33.9%	-
Range 9	83.2%	54.1%	42.2%	29.0%	-
Series II					
Range 1	40.2%	40.6%	59.8%	-	48.6%
Range 2	17.2%	34.1%	57.2%	-	45.4%
Range 3	32.3%	30.1%	49.8%	-	38.8%
Range 4	34.3%	28.8%	45.0%	-	35.9%
Range 5	38.9%	26.5%	34.3%	-	29.3%
Range 6	44.6%	25.8%	26.3%	-	25.4%
Range 7	45.5%	28.8%	21.1%	-	22.8%
Range 8	47.7%	28.0%	17.0%	-	21.0%
Range 9	51.4%	28.9%	14.9%	-	21.0%

Table 4. Decay coefficient values observed in experimental conditions and corrected to 20°C.

	Range 1	Range 2	Range 3	Range 4	Range 5	Range 6	Range 7	Range 8	Range 9
	Series I								
b^{26}	0.166	0.179	0.194	0.217	0.273	-	-	-	0.299
b^{20}	0.110	0.118	0.128	0.143	0.180	-	-	-	0.197
	Series II								
b^{30}	0.147	0.154	0.258	0.315	0.457	0.531	0.557	0.591	0.580
b^{20}	0.073	0.077	0.129	0.157	0.228	0.265	0.278	0.295	0.289

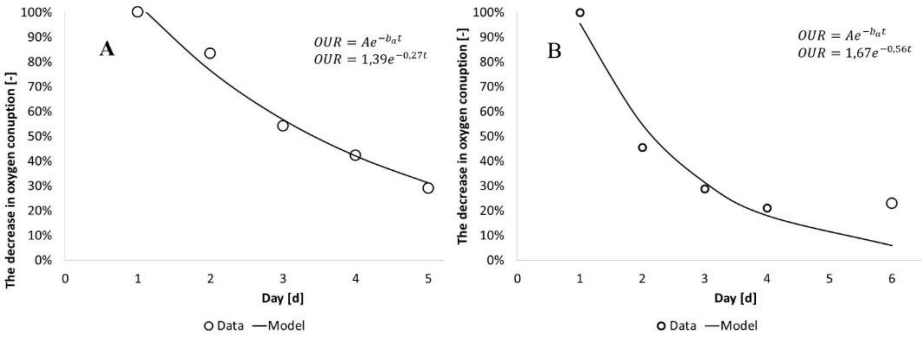

Fig. 4. Estimation results for A – range 9 (6.6 h) for Series 1, B – range 7 (9 h) for Series 2.

Analyzing the short test duration (ranges 1–3) substantial differences between experimental values and model predictions were observed. As mentioned before, such situation can be caused by slow adaptation of nitrifiers after starvation period, however no similar observation has been discussed in the literature so far. When test duration was extended (higher ranges in this analysis), calculated values was gradually approaching to the model predictions (fig. 5).

The best range for Series 1 & 2 (range 6 and range 7 respectively) was used in stage 2 of the analysis. In this stage first on or two first hours was cut from the database and new range were created: 10 and 11. Estimation results for new ranges were compared with previous ranges (Table 5).

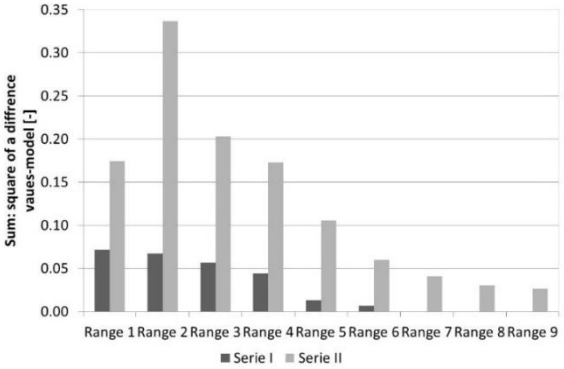

Fig. 5. Results of the estimation for all ranges in stage 1 of the analysis.

Table 5. Obtained results of decay rate for stage 2 of the analysis.

	Range 6	**Range 7**	**Range 10**	**Range 11**
Series I				
b^{26}	0.299	-	0.301	0.298
b^{20}	0.197	-	0.199	0.196
Difference between model and test (lower is better)	0.0067	-	0.0086	0.0152
Series II				
b^{30}	-	0.557	0.555	0.565
b^{20}	-	0.278	0.277	0.282
Difference between model and test (lower is better)	-	0.0408	0.0377	0.0356

Database modification performed in stage 2 (ranges 10 and 11) had no significant impact on decay coefficient calculations in comparison to previous ranges. That clearly indicates that respirometric assays used for decay coefficient estimation should be longer than several hours (c.a. 7 h or longer) and initial lag phase and low respirometric activity of the examined biomass has no relevant impact on the final result.

4 Conclusions

- Decay rate estimation using different methods is widely known topic. Decay rates determined for nitrifiers presented in literature are in the wide range of values, depending on biomass characteristics, experimental conditions and selected method of measurement
- Respirometric assays based on OUR measurements are an advantageous alternative for methods based on intensive chemical analysis (process kinetics observations) or molecular analysis (FISH, PCR) without significant decrease in the obtained results quality
- Performed data analysis revealed that short respirometric tests (up to 2 hours) resulted in unsatisfactory results comparing to model calculations. Low

respirometric activity, probably due to the lag phase after starvation period, affected the quality of experimental curve fitting to the existing decay model

- The best results were observed in long tests, which duration varied between 5–9 hours for both performed experimental series.
- Omitting the data from the observed lag phase (first 1–2 hours of respirometric assay) had no impact on the final results.

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References

1. M. Henze, W. Gujer, T. Mino, M. Loosdrecht, *Activated sludge models ASM1, ASM2, ASM2d and ASM3* (IWA, London, 2000)
2. B. Petersen, K. Gernaey, M. Henze, P. A. Vanrolleghem, Focus Biotechnol. **3C**, 101–186 (2003)
3. A. Lesouef, M. Payraudeau, F. Rogalla, B. Kleiber, Water Sci. Technol. **25**, 105–123 (1992)
4. J. Carrera, J. A. Torŕ, J. Lafuente, J. A. Baeza, Bioresource Technol. **102**, 9870–9875 (2003)
5. H. Yu, B. Ni, G. Sheng, Chem. Eng. Sci. **66**, 747–754 (2011)
6. S. Salem, M. S. Moussa, M. C. M. van Loosdrecht, Biotechnol. Bioeng. **94**, 252–262 (2006)
7. O. Nowak. K. Svardal, P. Schweighofer, Water Sci. Technol. **31**, 115–124 (1994)
8. H. Siegrist, R. Manser, W. Gujer, Water Res. **40**, 2416–2426 (2006)
9. J. A. Oleszkiewicz, G. Munz, C. Lubello, Water Res. **45**, 557–564 (2011)
10. J. A. Oleszkiewicz, G. Munz, C. Lubello, Chemosphere **83**, 720–725 (2011)
11. G. Tchobanoglous, F. L. Burton, H.D. Stensel, *Wastewater engineering: Treatment and Reuse / Metcalf & Eddy 4th edition* (McGraw Hill, New York, 2003)
12. S. Ge, S. Wang, X. Yang, S. Qiu, B. Li, Y. Peng, Chemosphere **140**, 85–98 (2015)
13. Y. Ahn, Process Biochem. **41**, 1709–1721 (2006)
14. S. Wyffels, S. van Hulle, P. Boeckx, E. Volcke, Biotechnol. Bioeng. **86**, 531–542 (2004)
15. N. Hubaux, G.Wells, E.Morgenroth, Water Res. **68**, 127–139 (2015)
16. J. A. Oleszkiewicz, S. A. Berquist, Water Res. **22**, 1163–1171 (1988)
17. H. Siegrist, I. Brunner, G. Koch, L. C. Phan, V. C. Le, Water Sci. Technol. **39**, 129–137 (1992)
18. J. A. Oleszkiewicz, Y. Lee, Water Res. **37**, 4202–4210 (2003)
19. R. Salzer, *A contribution to the determination of kinetic parameters for nitrification in activated sludge plants treating industrial wastewaters* (Institute for Water Quality, Viena, 1992).
20. E. J. T. M. Leenen, A. A. Boogert, A. A. M. van Lammeren, J. Tramper, R. H. Wijffels, Biotechnol. Bioeng. **55**, 630–641 (2000)
21. Z. Zhou, D. Hu, L. Jiang, C. Xing, Y. Zhu, M. Jiang, W. Qiao, Z. Li, Desalin. Water Treat. **56**, 2046–2054 (2015)

22. J. M. Choubert, A. Marquot, A. Stricker, S. Gillot, Y. Racault, A. Heduit, Water SA, **34** (2008)
23. P. L. Dold, R. M. Jones, C. M. Bye, Water Sci. Technol. **52**, 469–477 (2005)
24. D. Katehis, J. Fillos, L. A. Carrio, Water Sci. Technol. **46**, 289–295 (2002)
25. V. Martinage, E. Paul, Environ. Technol. **21**, 31–41 (2000)
26. E. I. P. Volcke *Modelling, analysis and control of partial nitritation in a SHARON reactor* (PhD thesis, Ghent University, Belgium 2006)
27. W. Liu, Q. Yang, B. Ma, J. Li, L. Ma, S. Wang, Y. Peng, Environ. Sci. Technol. **51**, 4001−4008 (2017)