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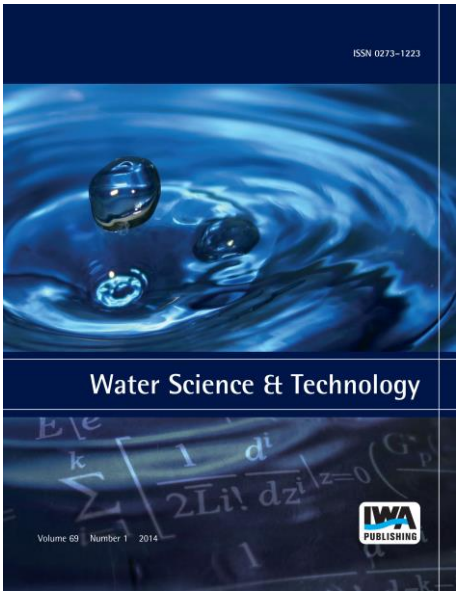

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Rethinking growth and decay kinetics in activated sludge – towards a new adaptive kinetics approach

Michael Friedrich, Jose Jimenez, Amy Pruden, Jennifer H. Miller, Jacob Metch and Imre Takács

ABSTRACT

Growth kinetics in activated sludge modelling (ASM) are typically assumed to be the result of intrinsic growth and decay properties and thus process parameters are deemed to be constant. The activity change in a microbial population is expressed in terms of variance of the active biomass fraction and not actual shifts in bacterial cellular activities. This approach is limited, in that it does not recognise the reality that active biomass is highly physiologically adaptive. Here, a strong correlation between maximum specific growth rate (μ_{max}) and decay rate (b_e) of ordinary heterotrophic organisms was revealed in both low solids retention times (SRT) and high SRT activated sludge systems. This relationship is indicative of physiological adaptation either for growth (high μ_{max} and b_e) or survival optimization (low μ_{max} and b_e). Further, the nitrifier decay process was investigated using molecular techniques to measure decay rates of ammonia oxidizing bacteria and nitrite oxidizing bacteria over a range of temperatures. This approach revealed decay rates 10–12% lower than values previously accepted and used in ASM. These findings highlight potential benefits of incorporating physiological adaptation of heterotrophic and nitrifying populations in future ASM.

Key words | activated sludge, decay rate, growth rate, high rate treatment, nitrification, physiology

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INTRODUCTION

Growth characteristics of ordinary heterotrophic organisms (OHOs) as well as ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB) are of particular interest for modelling activated sludge systems. However, quantification of microbial growth and decay in activated sludge is hindered by the complexity of a mixed microbial composition and an extreme diversity of substrates with various degradation characteristics.

OHOs represent the vast majority of active biomass in activated sludge. They are responsible for organic material degradation resulting in biomass production and, therefore, their growth characteristics greatly impact the solids retention times (SRT). The rate at which excess biomass is produced is also affected by the decay rate of these organisms. On the other hand, comparatively slow growing organisms, such as autotrophic AOBs and NOBs, greatly impact the minimum SRT because of their very low net growth rates.

Due to continual cycles of feast and famine in a water resource and recovery facility (WRRF) and the extreme variation in nutritional states as a result of very low (e.g., 0.5 d) and very high (e.g., 100 d) SRT, both growth and decay heavily impact the activated sludge process. However, with respect to kinetics, the magnitude of the active biomass fraction in high SRT activated sludge systems is less dependent on the maximum specific growth rate (μ_{max}) and more dominated by the decay rate (b_e). This is because the doubling time of the organisms is much lower than the system's SRT and it is usually sufficient to describe growth in terms of biomass yield in a stoichiometric fashion. In contrast to that, in very low SRT systems, μ_{max} becomes an important parameter and decay has less impact for the prediction of the active biomass fraction. However, modelling of microbial growth in high rate activated sludge (HRAS) systems where hydrolysis is already supressed and the storage response possibly reduced is still in discussion (Jimenez *et al.* 2015).

Historically, the growth kinetics of bacteria in activated sludge have been assumed to be governed by intrinsic properties. This assumption has largely been based on pure

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culture studies under ideal conditions. Although the range of μ_{max} and b_e in the literature is very large, both fundamental growth parameters in widely used activated sludge models (ASMs) are fixed to typical mean values (Henze *et al.* 2000). As a result, this static approach to microbial growth kinetics in recent ASMs expresses microbial activity only in terms of the magnitude of active biomass and not in terms of the activity of the individual bacterial cells. On this basis, in the model a bacterial cell works without metabolic adaptation, or in other words the bacterial cell can only be switched on by growth and switched off by decay and cannot vary its fixed intrinsic growth properties.

The *status quo* of modelling growth for a microbial population using only a mean value for growth parameters while ignoring the physiology of bacterial cells is not representative of reality. Bacteria, by nature, are masters at adapting their physiology and metabolism in the face of changing environmental conditions. Genetically predefined intrinsic properties of bacteria, as can be found in pure cultures, change in response to environmental stress outside the ideal conditions of the laboratory. There are numerous such stress factors at play in WRRFs (availability of electron acceptor, temperature, pH, inhibitors, salt concentration, etc.). However, the most prominent factors are the feast and famine pattern, which is thought to be responsible for the storage response of OHO (Majone *et al.* 1999), as well as the historical nutrient supply that is numerically expressed in terms of SRT. These stress factors shape the physiological state and ultimately the ecological structure (diversity, composition) of activated sludge microbial communities.

Physiological adaptation of growth kinetics has been intensively discussed in earlier literature (Daigger & Grady 1982; Grady *et al.* 1996). Recent research confirms the existence of mechanisms that lead to an adjustment of the macromolecular composition of the bacterial cell; and therefore, the adjustment of kinetic properties when the population is exposed to changing environmental factors (Lavallée *et al.* 2005; Pala-Ozkok *et al.* 2013).

A critical discussion of a constant growth kinetics approach is required in the light of the increasing importance of HRAS systems as well as low sludge production processes like the Cannibal™ process. But it can be expected that even for medium SRT systems, often applied in engineering practice, an adaptive growth kinetic approach would be a more realistic explanation of microbial life.

This study merges kinetic data from a pilot-scale HRAS system with low SRTs from 0.5 to 2.0 d (low SRT study) and data from different WRRFs with high SRTs ranging from 11 to 70 days (high SRT study) and demonstrates that both μ_{max} and b_e are influenced by the physiology of OHOs. Moreover, it is further demonstrated that these kinetic parameters can be linked to each other, which could form the basis for a more consistent and adaptive kinetic approach in a future ASM. Furthermore, decay rates of autotrophic nitrifiers, which represent a more homogenous group than OHO, are often even more oversimplified than heterotrophic growth and decay processes in activated sludge. A critical examination of autotrophic decay is of particular interest because of the impact on the sizing of the activated sludge process.

IDENTIFYING HETEROTROPHIC KINETIC GROWTH PARAMETERS

Decay rate (b_e)

Decay is known as the reduction of heterotrophic biomass (X_{OHO}) due to various causes, where the most prominent is endogenous respiration. The decay rate is measured by aerobic degradation batch experiments. It is common practice to indirectly measure decay rates as the exponential decrease of the biodegradable organic material (VSS_{DEG}) or endogenous oxygen uptake rate (OUR) (Ramdani *et al.* 2010). It is believed that this decay process is active even in the presence of external substrate.

Problems arise for the VSS method because it depends on a fixed non-biodegradable organic fraction (VSS_U), since VSS_{DEG} approaches VSS_U asymptotically. However, recent research has shown that VSS_U is not as non-biodegradable as was thought for a long time (Spérandio *et al.* 2013). If VSS_U decreases during an aerobic degradation batch experiment, the line to approach $VSS_{DEG} = 0$ would be uncertain and therefore the value for the decay rate too high.

The OUR method, on the other hand, although simple in approach, requires a high accuracy of OUR measurements. Oxygen intrusion via an open water surface would result in an overestimation of the decay rate. Additionally, the use of allylthiourea to suppress oxygen consumption due to nitrification can significantly increase the decay rate estimate (Friedrich 2016).

By analysing respirograms from activated sludge samples from WRRF with different SRTs, it is apparent that the exponential OUR decrease is not as homogenous as originally thought (Friedrich & Takács 2013). In

particular, a significantly faster OUR decrease was detected at the beginning of the degradation experiment. It was assumed that this rapid initial OUR decrease was partly caused by the degradation of stored organic material X_{STOR}. By excluding the initial X_{STOR} degradation from the endogenous respirogram, the X_{OHO} decay rate showed a more adaptive characteristic with respect to the physiology of X_{OHO}. This was demonstrated by comparing the initial specific $OUR_{OHO}(0)$ (which is highly indicative for the systems SRT) to the decay rate b_e, revealing a strong correlation of both parameters ($R^2 > 0.95$).

Maximum specific growth rate (μ_{max})

Two substantially different approaches are widely used to determine the maximum specific growth rate depending on the substrate (S) to biomass (X) ratio employed during the bioassay:

High S/X method with wastewater

This method was proposed by Kappeler & Gujer (1992) and is frequently used in research and practice. It was designed to measure the maximum specific growth rate for X_{OHO} as well as the readily biodegradable fraction of the chemical oxygen demand (COD) in wastewater. The S/X ratio in this method is mainly higher than 20 mg COD_S/mg COD_X. Wastewater or an appropriate mixture of wastewater and activated sludge is aerated in a batch reactor and the OUR is measured. The maximum specific growth rate can be derived from linearization of the exponential increase of OUR. However, two aspects of this procedure are critical:

(1) The experimental conditions of a high S/X ratio do not accurately reflect the low S/X conditions typically present in activated sludge systems, thus the physiological state of the bacterial cell as well as the adaptation to stress factors the bacterial cell is exposed to in the WRRF are not considered.
(2) Even if this method is based on the detection of an exponential increase of OUR and therefore can be related to an exponential growth, it is likely at such high S/X ratios that the growth yield coefficient Y is already reduced (Chudoba et al. 1992). For example, a reduction of Y from 0.66 down to 0.46 mg COD_S/mg COD_X would double the term (1 – Y)/Y. In fact comparing the maximum specific growth rates from both the low and the high S/X ratio method in the literature shows higher μ_{max} for the high S/X ratio method.

Low S/X method with activated sludge

This method is extensively described by Ekama et al. (1986) and has been applied and modified by many researchers since. The low S/X method assumes that $\mu = \mu_{max}$ after wastewater is spiked into activated sludge. The observed plateau of maximum OUR represents the maximum specific growth rate. For this method, the S/X ratio was typically smaller than 0.5 mg COD_S/mg COD_X. The advantage is that μ_{max} is derived under conditions that are very similar to the inlet of a WRRF where wastewater meets activated sludge. The immediate OUR response is genuine with respect to the wastewater treatment process and it recognises the physiological state and therefore the culture history of the bacteria cell. Furthermore the stability of the OUR plateau proves that substrate saturation is achieved and would lead to $\mu = \mu_{max}$. But again, there are critical aspects associated with this method:

(1) Since μ_{max} depends on X_{OHO}, it is based on several model parameters and its accuracy is only valid in the environment of this particular model.
(2) This method does not show an exponential increase of OUR as typical and necessary for unlimited exponential growth as described by Monod (1949).

Low S/X method by combining degradation and growth experiments

To avoid the need of knowing the active biomass concentration of X_{OHO}, Friedrich et al. (2015) suggested a procedure to determine μ_{max} by combining degradation and growth experiments. From the degradation batch experiment, the decay rate b_e was derived as described. X_{OHO} at the beginning of the batch test can be estimated from:

$$X_{OHO}(0) = \frac{OUR_e(0)}{(1 - f_U) \cdot b_e} \quad (1)$$

where f_U is the endogenous residue fraction of X_{OHO} and the index e refers to an endogenous process.

A growth experiment with the same activated sludge was conducted, where growth was induced by spiking the sludge with acetate in excess. The growth of X_{OHO} at the moment of spiking (t = 0) can be described in terms of OUR by recognizing growth and decay simultaneously according to Equation (2):

$$OUR_{max}(0) = \frac{(1 - Y)}{Y} \cdot \mu_{max} \cdot X_{OHO}(0) + (1 - f_U) \cdot b_e \cdot X_{OHO}(0) \quad (2)$$

Since X_{OHO} in both batch tests is the same, Equation (1) can be introduced into Equation (2), which yields an expression according to Equation (3) for μ_{max}.

$$\mu_{max} = \frac{Y}{(1-Y)} \cdot (1 - f_U) \cdot b_e \cdot \left(\frac{OUR_{max}(0)}{OUR_e(0)} - 1 \right) \quad (3)$$

Using this equation, the determination of the μ_{max} relies mainly on measured parameters, namely OUR_{max} after spiking from the growth experiment, the initial endogenous OUR_e exclusively performed by X_{OHO} from the degradation experiment, as well as the decay rate b_e. The growth yield Y and the endogenous residue fraction f_U are reliably well defined default values for activated sludge (Ramdani *et al.* 2010).

LINKING HETEROTROPHIC GROWTH AND DECAY RATES

Relation of μ_{max} and b_e

Recognizing the importance of physiological adaptation to environmental stress, in particular to the nutritional state of the bacteria cell in activated sludge, it can be expected that kinetic growth and decay properties should display similar trends. Indeed, combining degradation and growth experimental results for μ_{max} and b_e from low-rate activated sludge systems (Friedrich *et al.* 2015), as well as high-rate activated sludge data (unpublished data from Jimenez *et al.* 2015), yields a strong dependency of both growth parameters (Figure 1). This positive correlation for mixed culture populations like activated sludge is consistent with pure culture studies (Neijssel & Tempest 1976), where an analogous linear relationship between endogenous respiration in a carbon limited medium and the dilution rate was described for pure cultures of *Klebsiella aerogenes*.

However, to approach a unifying theory of adaptive growth kinetics beyond the high SRT data from Friedrich *et al.* (2015), it is important to consistently extend the characteristic relation of μ_{max} and b_e to very short SRTs. Considering these findings, a variable and adaptive growth kinetics approach seems to more realistically describe growth kinetics in activated sludge.

Physiological state factor

The physiological classification of activated sludge could help to introduce the adaptability of μ_{max} and b_e into activated sludge modelling, which so far has been neglected. Due to the strong correlation of both growth parameters, it is ideal to use the ratio of μ_{max} and b_e as indicative of the physiological state of OHO in the activated sludge (Friedrich *et al.* 2015). This ratio was named the physiological state factor (PSF):

$$PSF = \frac{\mu_{max}}{b_e} \quad (4)$$

The high and low SRT data shown in Figure 2 illustrate the good correlation of PSF with the SRT based on an exponential function. Thereby, a high PSF describes a microbial community that is growth optimized and a low PSF refers to a population that is survival optimized. To illustrate how this new perspective of microbial growth behaviour contrasts to existing ASMs, the PSF of ASM3, which is 8.3 ($\mu_{max} = 2\ d^{-1}$ and $b_e = 0.24\ d^{-1}$), is inserted into Figure 2 and represented as the dashed line. However, as a consequence of Equation (3), the ratio of $OUR_{max}(0)/OUR_e(0)$ generates different values for PSF, but would result in the same function.

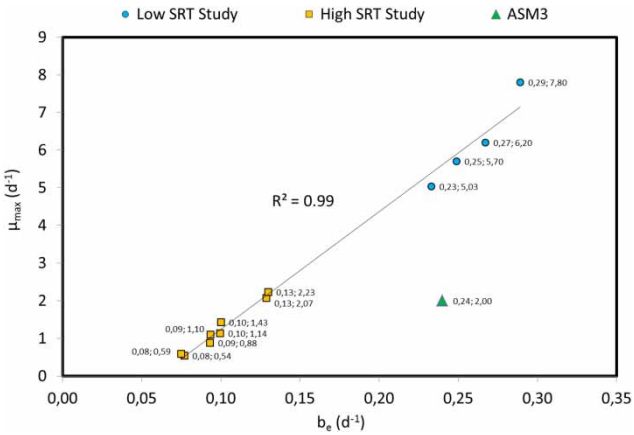

Figure 1 | Relationship between μ_{max} and b_e.

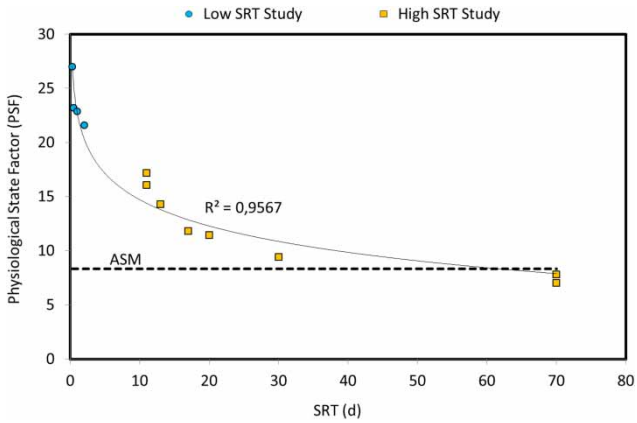

Figure 2 | PSF as a function of SRT.

KINETICS OF NITRIFYING ORGANISMS

Nitrifier growth kinetics are crucial for sizing aeration basins in activated sludge processes where nitrification is required. However, nitrifier growth and decay parameters have not been as well characterized as OHO parameters. This is due to the determination of the nitrifier maximum specific growth rate, which requires knowledge of the nitrifier decay rate. Otherwise, quantifying the nitrifiers decay rate demands an estimate of OHO concentration because decayed OHO serves as nitrogen source and, therefore, delays the decay of nitrifiers in starving activated sludge. Furthermore, the nitrifier decay rate is historically defined as a loss in nitrification activity measured in nitrate production rate (NPR) batch tests without consideration of nitritation activity. As such, the same growth characteristics have been historically assigned to both AOBs and NOBs.

Due to this structural and methodical uncertainty, it is promising to combine traditional activity measurements like NPR with molecular techniques like quantitative polymerase chain reaction (QPCR) of DNA markers to gain insight into decay mechanisms of AOBs and NOBs and their kinetics as a function of temperature. In this study, the traditional NPR batch test was modified to enable determination of both nitritation and nitrification activity, thus separate decay rates for AOBs and NOBs were calculated. In addition, QPCR was used to measure the decay of DNA markers associated with AOBs and NOBs. Unfortunately, growth parameters were not measured in this study because of time and cost constraints.

RESULTS

NPR assays conducted over a range of temperatures (15 °C–27 °C) revealed that previous models have overestimated the sensitivity of nitrifier decay rates to temperature changes (see ASM2/3 Θ default values in Table 1 (Henze *et al.* 2000)). This has a significant design effect at low temperatures, because reactor sizes are based on the lowest expected temperature where nitrification is required. It is worth noting that decay rates used in ASMs may also be too high (see WERF default b values in Table 1 (WERF 2003)). A reduced AOB decay parameter relative to the default model value would predict a smaller peak ammonia concentration in plant effluent in response to high ammonia loads. Further, DNA markers decayed at an even slower rate than the corresponding activity decay (Pruden *et al.* 2014). The latter may be evidence of nitrifiers entering a dormant state in which the cell is intact and DNA is preserved within the cell, but the cells are not metabolically active.

Table 1 | Decay rates and temperature coefficients of nitrifiers from WERF (2016)

				This study			
				AOB		NOB	
	Unit	ASM2/3 Nitrifier	WERF Nitrifier	Activity	DNA	Activity	DNA
b_{20}	d^{-1}	0.150	0.170	0.136	0.072	0.089	0.077
b_{10}	d^{-1}	0.050	0.128	0.108	0.034	0.062	0.044
Θ	–	1.116	1.029	1.023	1.079	1.036	1.057

As a consequence of lower decay rates at low temperatures, it may be possible to reduce the minimum SRT by 10–12% when using newly measured decay rates and temperature correction coefficients, as compared to existing default values. Although at this stage we do not yet recommend that design SRTs be reduced 10–12% in practice, this result reveals the need to investigate growth rates, decay rates and their temperature sensitivities further to potentially reduce safety factors or use revised decay rates and temperature corrections for reduced reactor sizing and significant cost savings.

In addition, decay rates are essential for maximum specific growth rate determination of nitrifiers, thus this research direction is important towards improving general understanding of growth kinetics of nitrogen oxidation processes. Although maximum specific growth rates of nitrifiers were found to be high in low SRT systems and low in high SRT systems (WERF 2003), little is known about their physiology and adaptation to environmental changes. The methodology and the results proposed and explored here can contribute to more precise modelling of activated sludge systems in the future, which is critical for optimizing process performance and efficiency.

CONCLUSION

The *status quo* of assuming growth kinetics based on static intrinsic properties as obtained from pure cultures under undisturbed and substrate rich laboratory conditions is not reflective of the reality of wild-type microorganisms as they exist in activated sludge. Physiological adaption is a fundamental, complex and significant strategy that microorganisms employ to optimize growth and survival in natural and engineered environments. Here it was demonstrated that measured maximum specific growth rates and decay rates for OHOs are shaped by such microbial adaptive strategies. Incorporation of an adaptive growth kinetics approach would predict a higher

fraction of OHOs in activated sludge, especially at high SRTs, than predicted based on the default ASM assuming non-adaptive growth kinetics. However, this higher active fraction would have a lower activity compared to the active fraction of a low SRT system. Furthermore, considering carbon capture with extremely low SRT systems followed by a nitrifying and therefore higher SRT system, an adaptive growth kinetics approach will be useful to describe different microbial consortia.

Since physiological adaptation changes μ_{max} and b_e in a similar way, the ratio of the two parameters expressed as PSF is promising for developing an improved activated sludge model concept.

The nutritional state of the bacterial cell and the magnitude of all the processes that are responsible for microbial decay are important to understand physiological adaptation. However, the decay of autotrophic nitrifiers is a process that is still poorly understood and needs to be described more in detail. Improved nitrifier decay rates will enhance estimates of the presence and amount of active biomass, which becomes especially important under operating conditions at critically low temperatures or high ammonia loads.

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