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Physiological adaptation of growth kinetics in activated sludge

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ABSTRACT

Physiological adaptation as it occurs in bacterial cells at variable environmental conditions influences characteristic properties of growth kinetics significantly. However, physiological adaptation to growth related parameters in activated sludge modelling is not yet recognised. Consequently these parameters are regarded to be constant. To investigate physiological adaptation in activated sludge the endogenous respiration in an aerobic degradation batch experiment and simultaneous to that the maximum possible respiration in an aerobic growth batch experiment was measured. The activated sludge samples were taken from full scale wastewater treatment plants with different sludge retention times (SRTs). It could be shown that the low SRT sludge adapts by growth optimisation (high maximum growth rate and high decay rate) to its particular environment where a high SRT sludge adapts by survival optimization (low maximum growth rate and low decay rate). Thereby, both the maximum specific growth rate and the decay rate vary in the same pattern and are strongly correlated to each other. To describe the physiological state of mixed cultures like activated sludge quantitatively a physiological state factor (PSF) is proposed as the ratio of the maximum specific growth rate and the decay rate. The PSF can be expressed as an exponential function with respect to the SRT.

1. Introduction

The activated sludge process is one of the most widespread microbial mixed culture-applications in an engineered environment. Therefore, an extensive knowledge of microbial growth kinetics is essential for a high quality process design and operation, in particular for the prediction of sludge composition and sludge production as well as oxygen consumption.

For practical reasons activated sludge models (ASMs), irrespective of whether they are complex dynamic or simple steady state models, consider bacteria not as individual organisms. In ASMs the mass of bacteria cells (X) is modelled as a major organic mass fraction of volatile suspended solids (VSS) and grouped with respect to their metabolism and function within the activated sludge process. ASMs transfer growth related characteristic properties of the bacterial cell to the mass fraction of the particular organism group.

Growth kinetics of microbial life in ASMs is based on the work of Monod (1949) describing the growth of bacteria in pure cultures on the utilisation of single substrates. This involves a mathematical saturation expression for the specific growth rate of bacteria (Eq. (1)) depending on a specific maximum growth rate (μ_{max} in d^{-1}), a growth limiting substrate concentration (S in mg/l) and a substrate affinity (K_S in mg/l):

$$\mu = \mu_{max} \cdot \frac{S}{K_S + S} \quad \left(d^{-1}\right) \tag{1}$$

where μ_{max} and K_S are parameters describing the characteristic growth properties of a certain bacteria species.

Furthermore, Monod (1949) identified a constant relationship of biomass growth and limiting substrate utilisation. The resulting stoichiometric parameter, the yield coefficient Y (in mg COD_X/mg COD_S), is characteristic for a particular substrate and therefore reflects the substrate and subsequent energy conversion of the metabolism of a bacterial cell. In activated sludge modelling the yield coefficient has a major impact on the prediction of sludge production and oxygen consumption, whereas the maximum specific growth rate governs the oxygen utilisation rate in activated sludge systems.

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To explain the observed sludge production in an activated sludge system a growth antagonistic process had to be recognised (Herbert, 1958). This process was termed decay and its metabolic explanation comprises all possible ways of reduction of active microbial biomass like endogenous respiration (Gujer et al., 1999), death-regeneration (Dold et al., 1980), maintenance (Loosdrecht van and Henze, 1999) or predation (Moussa et al., 2005). The kinetics of the decay process was identified and modelled as first order reaction with respect to bacterial biomass (Marais and Ekama, 1976). The decay rate constant b (d^{-1}) was thought to be independent of the substrate supply and SRT of the activated sludge, respectively (van Haandel et al., 1998). Considering an aerobic decay rate constant of 0.24 d^{-1} (Ramdani et al., 2010) this parameter has a significant impact on the observed sludge production, since independent of the substrate supply and therefore independent of the growth situation 24% of microbial biomass is degraded per day. In fact, it is the decay rate parameter within the conceptual framework of an ASM that is most important for the existence and magnitude of the active biomass fraction in activated sludge over the possible range of SRTs.

As their constancy implies the key parameters of microbial growth kinetics in ASMs namely μ_{max} and b are regarded to be "intrinsic" with regard to the organism group. In this way they are independent of the culture history with respect to variations in substrate supply. Therefore, growth kinetics as expressed in ASMs is not subjected to a physiological adaptation as it occurs in nature to bacterial cells. To demonstrate the drastic consequences of a constant growth kinetic approach the applied range of SRT as recommended for the use of ASMs (3–20 d) can be expanded:

For a high loaded sludge from a low SRT system (e.g. SRT = 1 d) a high active biomass fraction of 80% will be predicted. On the contrary for a starving sludge from a high SRT system (e.g. SRT = 50 d) an ASM calculates a low active biomass fraction of less than 20%. As a modelling result both systems differ significantly in their composition of constituents, in particular their active fractions, but the physiological properties of the actors namely the bacterial cells in both systems are still the same.

Additionally, the constancy of μ_{max} further implies that any bacterial cell will maintain the potential to exhibit the maximum growth rate as soon as the substrate concentration reaches saturation. That means bacterial cells from a low SRT system will show the same μ_{max} as cells from a high SRT system. Consequently, in terms of respiratory activity a long term starved bacterial cell would have the same maximum oxygen utilisation rate (OUR_{max}) as a bacterial cell from a high loaded system.

To illustrate that further, the specific OUR_{max} at substrate saturation can be calculated from Eq. (2) (Herbert, 1958; McKinney, 1960). It considers oxygen consumption due to substrate utilisation resulting in microbial growth and oxygen consumption due to endogenous respiration.

$$\frac{OUR_{max}}{X_{OHO}} = \frac{(1 - Y_{OHO})}{Y_{OHO}} \cdot \mu_{max,\ OHO} + (1 - f_U) \cdot b_{OHO} \quad \text{mg } O_2/(\text{mg } X_{OHO} * \text{d}) \tag{2}$$

Using default values (WRC, 1984) of ordinary heterotrophic organisms (X_{OHO}):

Y_{OHO} = 0.67 g COD/g COD,
$\mu_{max,OHO}$ = 2.0 d^{-1},
b_{OHO} = 0.24 d^{-1},
f_U = 0.2 as the endogenous residue fraction

the specific OUR_{max} performed by any modelled ordinary heterotrophic bacterial cell in activated sludge in the presence of excess substrate is a constant value of 1.17 mg O_2/(mg X_{OHO}*d). It is unlikely that a constant respiratory potential reflects the reality of microbial life in activated sludge, because in that case a starving cell would have the same cellular equipment as a cell grown in an environment with excess substrate. But there are further aspects indicating the need for a critical discussion of the deficiencies of constant growth kinetics:

First, the recommended parameter sets for growth kinetics are obtained for a medium range of SRTs, which comprises SRTs from 3 to 20 days (Henze et al. (1987, 1995); Gujer et al. (1999)). Today there are extremely high loaded activated sludge systems in operation like the AB- process for optimized energy conservation with SRTs <1 day (A-stage) and extremely low loaded systems with SRTs >50 days like the OSA (Chen et al., 2003) or Cannibal process (Novak et al., 2006) for the minimization of excess sludge production.

Second, in the literature the range of values reported for maximum specific growth rates (Table 1) as well as decay rates (Friedrich and Takács, 2013) is so large that in general the value of one of these kinetic parameters can only be used in the context of the culture history of the samples and the bioassay that produced these values. In particular there is the tendency that for a high ratio of substrate to active biomass (S/X ratio) within the determination procedure μ_{max} has a high value and low S/X ratios produce rather low μ_{max} values.

Third, reviewing the literature there is an extensive knowledge addressing physiological adaptation of bacterial cultures. In a historical perspective Jannasch and Egli (1993) describes the metabolic control in the course of the culture history as a "new dimension to growth kinetics". In this context the volume "Starvation in bacteria" (Kjelleberg, 1993) is worth mentioning as a combination of important research papers addressing physiological changes of bacteria under changing environmental conditions in particular under starvation.

These references are primarily microbiological research papers dealing with pure culture studies. However, researchers in engineering science are also aware of the need to introduce variable growth kinetics into activated sludge modelling. In particular, the comprehensive work of Daigger and Grady (1982a, 1982b) and Grady et al. (1996) who discuss extensively the mechanisms of physiological adaptations in bacterial cells is of high value for a deeper understanding of the dynamics of growth kinetics. Lavallée et al. (2005) suggests a comprehensive model recognising the metabolic adaptation of biomass under different growth conditions. Orhon et al. (2009) presented experimental evidence for a variable growth kinetics by determining a high μ_{max} for a low SRT activated sludge and a low μ_{max} for a high SRT activated sludge calibrating ASM1 and ASM3 with data from a peptone degradation experiment. The more recent results of the study from Pala-Ozkok et al. (2013) using acetate as sole carbon source confirm that variable growth kinetics should be recognized in ASMs.

But there is still a lack of experimental data describing directly

Table 1
$\mu_{max,OHO}$ values for low and high S/X ratio methods.

	Low S/X	High S/X
	d^{-1}	d^{-1}
Kappeler and Gujer (1992)		7.5
Wentzel et al. (1995)		7.8
Sözen et al. (1998)		4.8
Nogaj et al. (2014)		7.0
Dold et al. (1991)	3.3	
Slade and Dare (1993)	1.5	
Pollard et al. (1998)	0.8	4.0

the physiological adaptation of growth kinetics of bacteria in activated sludge systems and the range in which their characteristic properties can vary is still uncertain. From the modelling perspective, it is of tremendous interest to know whether physiological adaptation is significant for the model results like oxygen consumption and sludge production or whether it is negligible.

To elucidate the physiological adaptation of $X_{OHO,}$ in this work the endogenous decay rate (b_e) is determined from endogenous respiration rate (OUR_e) profiles recorded in aerobic degradation batch experiments. Over the degradation time t_D of this experiment samples were taken in intervals and transferred to a separate reactor. In this reactor short term aerobic growth batch experiments were conducted by spiking the sample with excess substrate. From maximum possible respiration rate (OUR_{max}) the maximum specific growth rate μ_{max} can be derived. Additionally the reduction rate of the maximum growth potential (b_{max}) of X_{OHO} over the time of the aerobic degradation batch experiment can be obtained. By combining and comparing those two different measurements it is the aim of this study to find answers to the following questions:

- Is μ_{max} a true intrinsic parameter or does μ_{max} change in the course of the aerobic degradation batch experiment?
- Do endogenous (OUR_e) and maximum (OUR_{max}) respiration profiles correlate in the course of an aerobic degradation batch experiment?
- How are the kinetic properties of growth and decay influenced by physiological adaptation in activated sludge?

2. Materials and methods

This work is based on an extension of a previous study (Friedrich et al., 2015). A brief description of the used materials and methods will be given here.

2.1. Notation

This investigation deals exclusively with the kinetic growth properties of ordinary heterotrophic organisms (OHO). For reasons of simplicity the index "OHO" for the parameters Y, OUR, μ and b is omitted. For the characterisation of a parameter that was derived under endogenous conditions and therefore under metabolic stress where components of OHO are reduced the index "e" is used. Conditions of substrate saturation where OHO exhibit their maximum growth potential the index "max" is applied.

Furthermore instead of the long terms "aerobic degradation batch experiment" and "aerobic growth batch experiment" the shorter form "degradation test" and "growth test" is used, respectively.

However, it should be noted that the initial endogenous OUR of X_{OHO} termed $OUR_{OHO}(0)$ in Friedrich and Takács (2013) corresponds to $OUR_e(0)$ in this study.

2.2. Characteristic of activated sludge

Degradation tests were carried out for six different types of sludge taken from the return sludge of conventional single stage full scale activated sludge wastewater treatment plants (WWTP) situated in the Northeast of Germany ranging from 15,000 to 600,000 population equivalents (PE). All plants were fully nitrifying and performed an extensive denitrification. With the exception of plant C and D all WWTPs were equipped with a primary settler. Only plant E showed complete and plants A and F partial biological phosphorus removal. A basic classification of important parameters of the activated sludge samples being used in this investigation is presented in Table 2. The SRT corresponds to the mean SRT observed on the plant which is not as accurate as the SRT controlled in a lab scale plant.

2.3. Experimental setup

The experimental setup and the experimental approach are shown in Fig. 1. The degradation tests were conducted to observe the endogenous behaviour in the activated sludge samples in terms of OUR_e and VSS decrease over the time of the experiment. In particular the OUR_e measurements were used to determine the decay rate b_e of the heterotrophic organisms. Therefore OUR_e was measured in a closed reactor configuration (OUR_e-reactor) to exclude intrusion of oxygen via the water surface. To ensure the quality of the results a COD balance was performed for each aerobic degradation batch experiment. The COD recovery was regarded sufficiently high with values between 99.7% and 103.7%. The reactor temperature was controlled to 20 °C. The operation of the respirometer and the analytical methods are described in detail in Friedrich and Takács (2013).

The identical operated VSS-reactor was necessary to provide the sample volume for VSS determination as well as the inoculum for the growth tests. The extension of the former study consists of an OUR_{max}-reactor that was operated in parallel to the OUR_e- and the VSS-reactor for 1–2 days conducting the growth tests. The operation mode was such that in the first week every day and then in increasing intervals 500 ml inoculum from the VSS-reactor was withdrawn, diluted with effluent water from a WWTP and transferred into the OUR_{max}-reactor. A soluble organic substrate was spiked in the OUR_{max}-reactor in excess to display the maximum possible respiration potential of the aerobically digested activated sludge at the time t_D of the transfer from the VSS-reactor. However, the time of spiking is recognised as $t_G = 0$ with respect to the growth test. The OUR at this point is determined as $OUR_{max}(t_D,0)$. Acetate was used as substrate source, because it is most rapidly usable for heterotrophic organisms and the reproducibility of the experiments is much higher than with real wastewater. In that way acetate as substrate allows other researchers a better comparison to the results of this work.

The acetate dosage was variable. For tests A – C the dosage was such that the acetate concentration in the OUR_{max}-reactor was decreasing from the start of the test (100 mg COD/l) to the end (60 mg COD/l). The acetate dosage for the remaining tests was constant resulting in COD concentrations of 319 mg COD/l (test D) and 425 mg COD/l (test E and F).

2.4. Parameter determination

2.4.1. Decay rate of heterotrophic organisms (b_e)

The decay rate b_e is commonly measured as the exponential decrease of the endogenous respiration in terms of OUR in a degradation test excluding nitrification. This curve is linearized by plotting lnOUR versus degradation time and the slope of this line

Table 2
Characterisation of activated sludge samples.

Test	PE	VSS	VSS/TSS	OUR(0)	spOUR(0)	SRT
		mg/l	%	mg O_2/(l*h)	mg O_2/(g VSS*h)	d
A	200,000	2830	68	13.8	4.9	20
B	600,000	3020	74	20.3	6.7	11
C	80,000	3040	77	14.5	4.8	70
D	15,000	2663	80	25.8	9.7	13
E	50,000	2810	69	20.4	7.3	30
F	400,000	3060	77	17.5	5.7	17

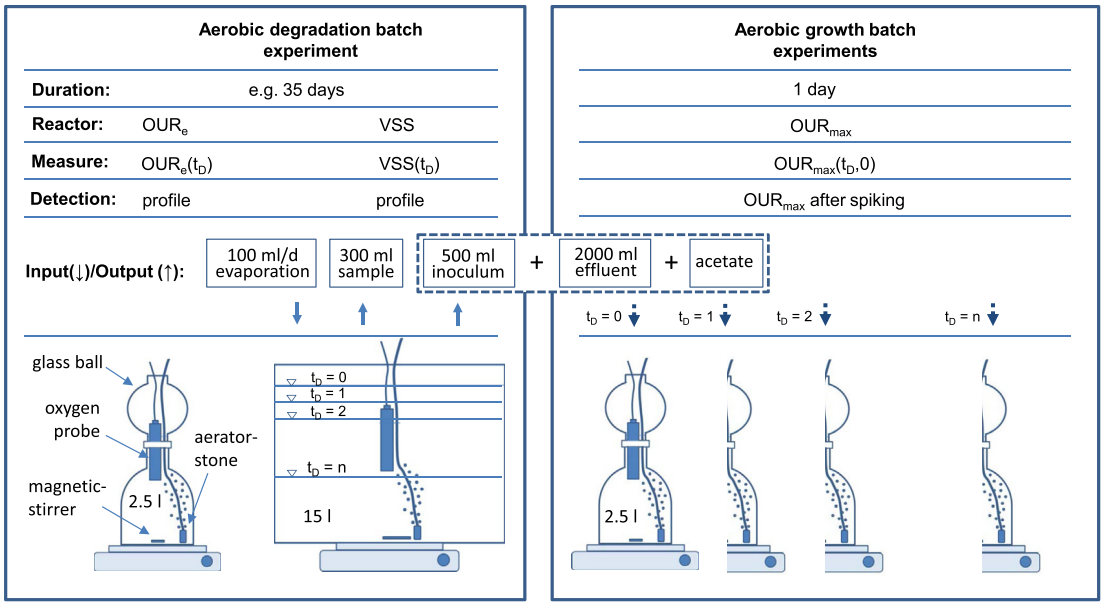

Fig. 1. Experimental setup.

yields the decay rate constant b_e.

However, Friedrich and Takács (2013) observed a rapid decrease at the beginning of a degradation test that did not match with a homogenous exponential decrease over the first 10–14 day. It was concluded that this rapid respiratory decrease was due to the degradation of stored material X_{STOR} which occurs simultaneously to the decay of X_{OHO}. Modelling both processes with a first order reaction kinetics yields a respirogram according to Fig. 2. The initial values of $X_{STOR}(0)$ and $X_{OHO}(0)$ as well as the rate parameter b_e and q_{STOR} were obtained by nonlinear regression parameter estimation. It is important to note, that the degradation of X_{STOR} is responsible for up to 50% of the initial total OUR, but represents only 2–5% of the degradable organic material in activated sludge. Therefore, without omitting the degradation of X_{STOR} the decay rate b_e would be estimated much higher. However, this procedure makes it possible to determine the initial endogenous respiration rate $OUR_e(0)$ of X_{OHO} for the degradation test, which is the starting point for a true exponential decrease of OUR_e and therefore the basis for the estimation of the decay rate parameter b_e.

2.4.2. Reduction rate of maximum growth potential b_{max}

During the degradation time $OUR_{max}(t_D,0)$ was decreasing. The rate of decrease is regarded as the reduction rate of the maximum growth potential b_{max}. The rate parameter b_{max} is obtained by linearizing the OUR_{max} values in the same way as for OUR_e over the time of the degradation test.

2.4.3. Maximum specific growth rate μ_{max}

For the determination of μ_{max} the indicative OUR_{max} after spiking the activated sludge with acetate was used (see Fig. 3). The added amount of acetate was selected to increase the substrate saturation term from the Monod kinetics to unity. At his point μ equals μ_{max}. The $OUR_{max}(t_D,t_G)$ in the growth test over the time of substrate saturation then is expressed with Eq. (3):

$$OUR_{max}(t_D,t_G) = \left[\frac{(1-Y)}{Y}\cdot\mu_{max}\cdot X_{OHO}(t_D,0)\cdot e^{\mu_{max}\cdot t_G} + (1-f_U)\cdot X_{OHO}(t_D,0)\cdot b_e\cdot e^{-b_e\cdot t_G}\right] \Big/ 24 \quad (mg\ O_2/(l^*h)) \tag{3}$$

This equation is based on Eq. (2) and therefore the assumption that decay does not stop or is reduced in the presence of excess substrate. Even if this is doubtful, it is also uncertain whether and how much the decay is reduced under these circumstances. With respect to aerobic degradability of X_{OHO} only a portion $(1-f_U)$ is degradable and therefore subjected to decay (Marais and Ekama, 1976; Ramdani et al., 2010). For the assessment of μ_{max} Eq. (3) is only of interest for $t_G = 0$, which is the time of spiking the activated

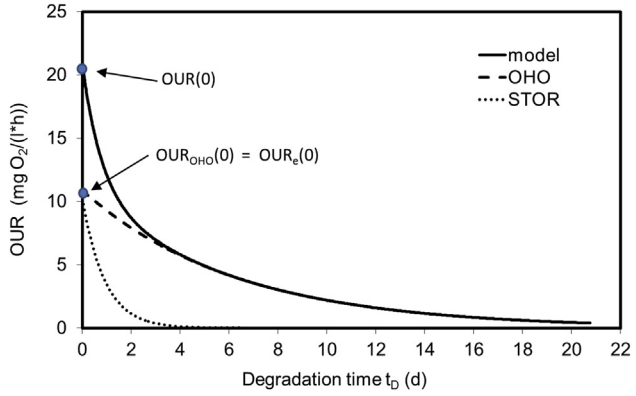

Fig. 2. Respirogram with the degradation of X_{STOR} and X_{OHO}.

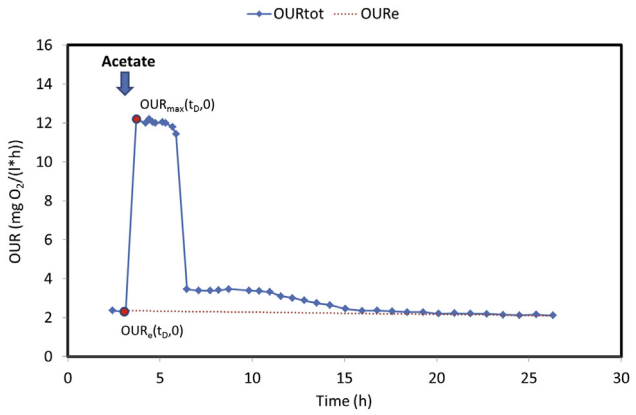

Fig. 3. Respirogram of an OUR_{max} experiment.

sludge with acetate. At the time of the first OUR measurement μ_{max} is already identifiable. For $t_G = 0$ Eq. (3) reduces to:

$$OUR_{max}(t_D,0) = \left[\frac{(1-Y)}{Y}\cdot\mu_{max}\cdot X_{OHO}(t_D,0) + (1-f_U)\cdot X_{OHO}(t_D,0)\cdot b_e\right] \Big/ 24 \quad (mg\,O_2/(l^*h)) \tag{4}$$

Rearranging Eq. (4) yields:

$$\frac{OUR_{max}(t_D,0)\cdot 24}{X_{OHO}(t_D,0)} = \frac{(1-Y)}{Y}\cdot\mu_{max} + (1-f_U)\cdot b_e \quad \left(d^{-1}\right) \tag{5}$$

However, $X_{OHO}(0)$ at the beginning of the growth test cannot be measured directly. But it can be calculated from $OUR_e(0)$ that is exclusively performed by X_{OHO} before the spiking of substrate and the decay rate b_e according to Eq. 6

$$OUR_e(t_D,0) = (1-f_U)\cdot X_{OHO}(t_D,0)\cdot b_e/24 \quad (mg\,O_2/(l^*h)) \tag{6}$$

Rearranging Eq. (6) for $X_{OHO}(t_D,0)$ yields

$$X_{OHO}(t_D,0) = \frac{OUR_e(t_D,0)\cdot 24}{(1-f_U)\cdot b_e} \quad (mg\ X_{OHO}/l) \tag{7}$$

Introducing Eq. (7) into Eq. (5) leads to:

$$\frac{OUR_{max}(t_D,0)\cdot(1-f_U)\cdot b_e}{OUR_e(t_D,0)} = \frac{(1-Y)}{Y}\cdot\mu_{max} + (1-f_U)\cdot b_e \left(d^{-1}\right) \tag{8}$$

Resolving Eq. (8) to μ_{max} gives an expression according to Eq. (9) that describes μ_{max} on the basis of the decay rate as well as the ratio of the endogenous and the maximum respiration rate of X_{OHO}.

$$\mu_{max} = \frac{Y}{(1-Y)}\cdot(1-f_U)\cdot b_e\cdot\left(\frac{OUR_{max}(t_D,0)}{OUR_e(t_D,0)} - 1\right)\left(d^{-1}\right) \tag{9}$$

The yield coefficient Y is determined as the integral of OUR between the endogenous $OUR_e(t_D,0)$ before and the $OUR_{max}(t_D,0)$ after substrate dosage with respect to the applied substrate COD.

It is important to note that within the experimental procedure used in this investigation only f_U has to be assumed, but this parameter is reliably estimated at $f_U = 0.2$ (Ramdani et al., 2010).

2.5. Presentation and repetition of experiments

To illustrate the dependency of growth and decay the results of degradation test B and C are displayed graphically (Figs. 4 and 5), whereas in summarizing considerations the results of all tests are discussed and presented in Table 3 and Figs. 6 and 7. In classifying the types of activated sludge with respect to SRT test B is used as representative for a low SRT sludge. Test C stands for a high SRT sludge. Since the values of the kinetic parameter of these two tests were extremes within the activated sludge samples tested in this study it was decided to repeat them and to include the results of these tests into the discussion.

3. Results and discussion

3.1. Endogenous versus maximum respiration rate profiles

The decrease of the endogenous respiration $OUR_e(t_D)$ with the rate of b_e is a measure for the decrease of X_{OHO}. In contrast to that, the decrease of the maximum respiration $OUR_{max}(t_D)$ indicates the loss of growth potential of X_{OHO} with the rate of b_{max}.

However, from measuring OUR_{max} alone it is not clear whether a high value of OUR_{max} is the result of a strong active biomass fraction with a low respiratory activity or rather the result of small active biomass fraction with a high respiratory activity.

From the OUR_e and OUR_{max} profiles (Fig. 4a and b) the rate parameter b_e and b_{max} were derived as described. According to Table 3 both rate parameter varied significantly. To characterise the variation of b_{max} over the time of the batch test it was necessary to classify them into 3 periods.

The rate of the loss of growth potential b_{max} for all tests with the exception of test A and C.2 was homogenous in the first two periods

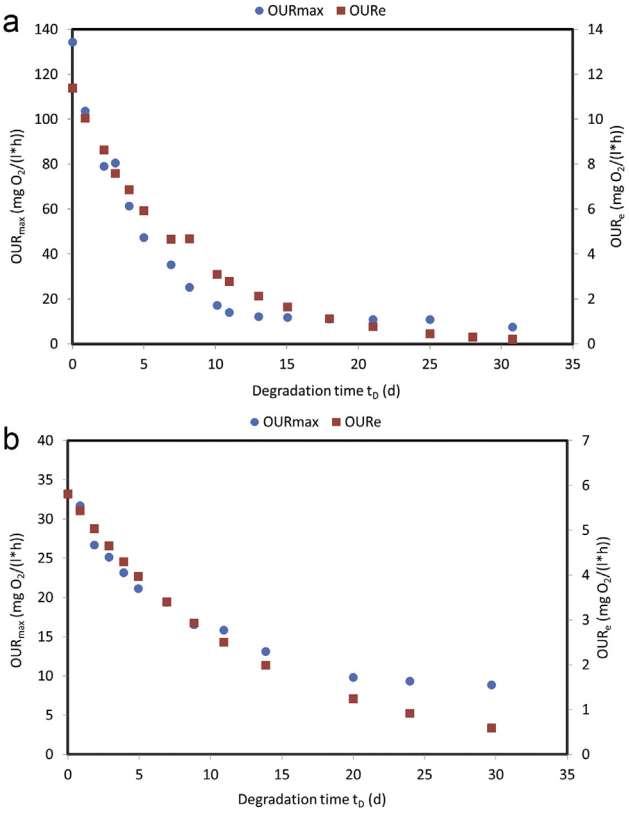

Fig. 4. a: OUR_e and OUR_{max} of test B.1. b: OUR_e and OUR_{max} of test C.1.

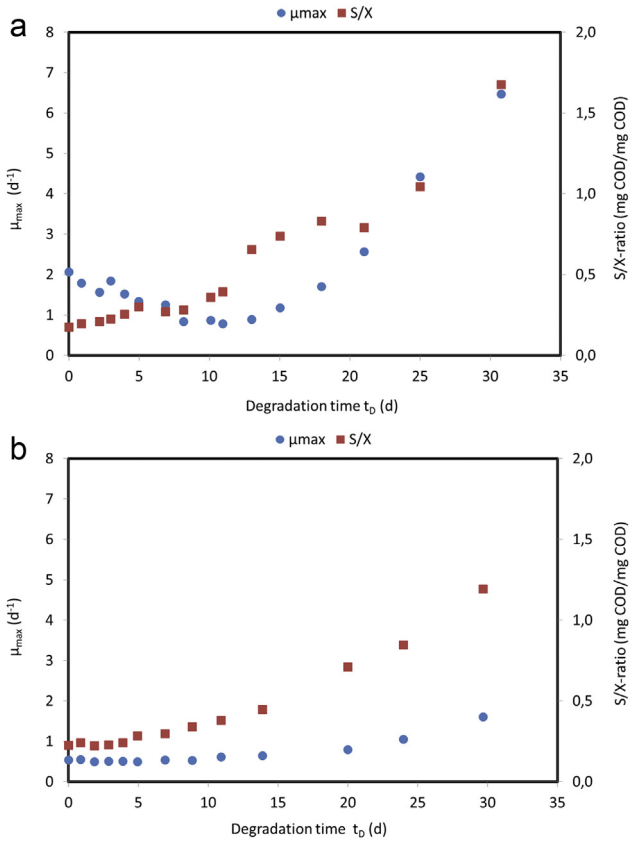

Fig. 5. a: μ_{max} and S/X-ratio of test B.1. b: μ_{max} and S/X-ratio of test C.1.

until t_a which is considered as adaptation time of X_{OHO} to starvation within the degradation test. Since b_e is determined with omitting the first rapid decrease of OUR due to the degradation of X_{STOR}, this observation supports the approach of Friedrich and Takács (2013) to exclude an initial high OUR_e for the determination of a more intrinsic decay rate b_e. Apparently, the organic material that is degraded in an early stage of starvation does not influence the kinetic properties of the heterotrophic biomass. In all tests b_{max} decreased significantly in the third period with respect to the first period and OUR_{max} could even increase. This characteristic in the third phase is similar as observed with b_e (Friedrich and Takács, 2013).

Comparing b_e with b_{max} in Table 3 shows that in general b_e is smaller for higher loaded sludge samples (test A, B.1, B.2, D, F) and both parameters are equal for lower loaded sludge samples (C.1, C.2, E).

With regard to culture history, it can be suggested that in absence of an external substrate source bacteria from high loaded systems adapt to starvation by reducing the cell internal machinery and therefore reducing the growth potential faster which is indicated by b_{max} that is higher than b_e. Whereas at low loaded systems adaptation already occurred within the culture history of the WWTP resulting in a low b_{max} that has the same low value as b_e and does not decrease further in the first periods of the degradation experiment.

An additional advantage of comparing endogenous and maximum respiration rates is the fact that OUR_e includes the respirational activity of microorganisms as well as higher organisms where OUR_{max} is supposed to display mainly the respiration of microorganisms, only. This would explain the different shapes of the OUR profiles in Fig. 4a from day 5–15 and assign an unexpected high respiratory activity to higher organisms, which in that particular case were an observed mass development of the rotifer Lecane.

However, since X_{OHO} is thought to be closely associated with the degradable organic material in activated sludge, its identifiability as an activated sludge component is based on the observation of endogenous respiration. Therefore OUR_e is more indicative for the magnitude of X_{OHO} than OUR_{max}.

3.2. Maximum specific growth rate profile

In the course of the degradation test measuring both the endogenous and the maximum OUR at the same time (see Fig. 4) and relating both values to each other generates the basis for the determination of μ_{max} according to Eq. (9). This implies furthermore that a variation of μ_{max} as documented in Fig. 5 corresponds directly to a variation of the ratio OUR_{max}/OUR_e. Notably, without physiological adaptation this ratio together with the decay rate b_e would be constant.

However, looking at the low SRT sludge of Fig. 5a the initial decrease of μ_{max} can be explained by a faster decrease of OUR_{max} relative to OUR_e within the first 11 days as indicated in Table 3. Surprisingly, after day 11 the ratio of OUR_{max}/OUR_e increases significantly showing that in spite of the further reduction of X_{OHO} the respiratory potential of the remaining organisms increases under conditions of substrate saturation.

In contrast to this, the high SRT sludge in Fig. 5b starts with a much lower μ_{max}. The decrease of μ_{max} until day 10 is very small since both b_e and b_{max} have nearly the same value. After day 10 again the ratio of OUR_{max}/OUR_e and consequently μ_{max} increases significantly. There are two possible explanations for this observation:

First, as indicated above after an adaptation time the remaining bacteria in the activated sludge exhibit a starvation survival

Table 3
Respiratory and kinetic results of degradation experiments (R^2 in brackets).

Test	Test-Time	OUR_{max} $t_D = 0$	μ_{max}	OUR_e	b_e	b_{max} P1 (<4 days)	P2 (t_a–4 days)	t_a	P3 (rest)
	d	mg O_2/l/h	1/d	mg O_2/l/h	1/d	1/d	1/d	d	1/d
A	49	72	1.139	8.3	0.100	0.233 (0.99)	0.085 (0.99)	18	0.035 (0.96)
B.1	31	134	2.067	11.4	0.129	0.201 (0.99)	0.201 (0.99)	11	0.013 (0.94)
B.2	42	158	2.232	12.6	0.130	0.165 (0.99)	0.165 (0.99)	14	0.032 (0.87)
C.1	30	33	0.540	5.8	0.077	0.078 (0.98)	0.078 (0.98)	10	0.031 (0.89)
C.2	22	29	0.585	4.6	0.075	0.081 (0.94)	0.055 (0.61)	14	0.049 (0.88)
D	42	96	1.430	9.0	0.100	0.139 (0.98)	0.139 (0.98)	16	0.049 (0.82)
E	76	60	0.878	8.2	0.093	0.090 (0.94)	0.090 (0.94)	16	increased
F	22	76	1.103	8.5	0.094	0.120 (0.99)	0.120 (0.99)	13	increased

response which becomes visible by OUR_{max} measurements. The equipment for the production of cell internal material of bacteria, in particular the number of ribosomes and the thickness of the cell membrane (Morita, 1993), is likely to be reduced during t_a to a minimum because of the lack of an external energy source. After t_a obviously some bacteria develop a starvation survival response by producing enzymes for the breakdown of more complex and therefore less biodegradable compounds in activated sludge. With finding an access to a new energy source it is possible to increase the cell equipment as it is observed by increasing OUR_{max} measurements relative to OUR_e.

Second, the S/X ratio might affect the increase of μ_{max}. In this context the S/X ratio is referred to as the initial substrate concentration in the batch reactor relative to the initial biomass concentration. It is noticeable that the S/X ratio increases due to a loss of X_{OHO} which might cause or just support the increase of μ_{max}. This explanation would be in a line with Chudoba et al. (1992) who could show for cultures exposed to low S/X ratios that the fade of substrate is mainly storage and less cell multiplication. On the contrary, at high S/X ratios the substrate is used exclusively for cell multiplication resulting in a high μ_{max}. However, the S/X ratios in the degradation experiments of this study are much smaller than those reported in the literature necessary for the induction of an increase of μ_{max}.

3.3. Maximum specific growth rate as an intrinsic property

From the experimental data of Fig. 5a and b it can be further concluded that the initial maximum specific growth rate $\mu_{max}(t_D,0)$ determined at the beginning of the degradation test is not a true maximum possible value in terms of an intrinsic property as it belongs to a certain genotype of bacteria. It expresses rather a maximum possible growth potential of a certain phenotype of bacteria that developed in the course of the culture history of the activated sludge.

The main factor within the environment of activated sludge forming the phenotype is certainly the nutrient supply of the bacteria. But not only the amount of nutrients expressed as S/X ratio is important, it might also be the pattern of nutrient supply (feast and famine) that will influence the physiological state of the bacterial cell (Kurland and Mikkola, 1993).

However, bacteria grown as pure cultures in a laboratory environment in rich medium and without environmental stress will exhibit physiological properties that are very close if not identical to the true definition of a genotype of the bacterial species (Kovárová-Kovar and Egli, 1998).

It is likely that in very high and very low loaded systems, respectively the physiological adaptation is outcompeted by or leads to the establishment of an adapted genotype that dominates the microbial community in activated sludge as observed by Pala-Ozkok et al. (2013).

3.4. Relation of μ_{max} and b_e

Looking at the initial values ($t_D = 0$) of μ_{max} and b_e (Fig. 6) which are characteristic for the activated sludge as it was taken from WWTP there is a strong correlation between these two seemingly antagonistic parameters. This correlation confirms that the observations of Kurland and Mikkola (1993) for pure cultures are valid even for mixed cultures.

An explanation can be formulated as follows: In an engineered environment like activated sludge bacteria in a high loaded system base their survival on a growth rate maximization on the expense of a high decay rate, whereas bacteria in a low loaded system improve their survival by one or more mechanisms of self-protection to reduce the decay rate on the expense of a low maximum growth rate.

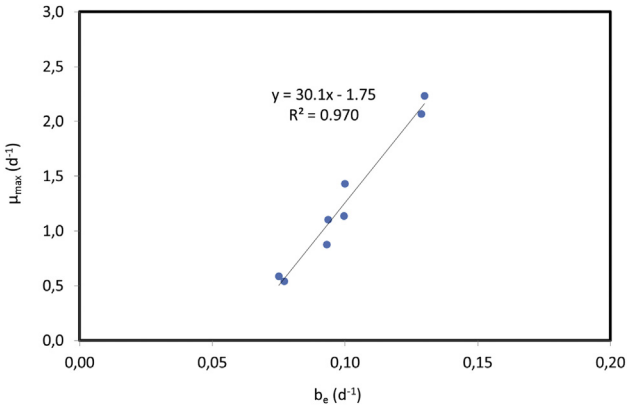

Fig. 6. Relation of μ_{max} and b_e.

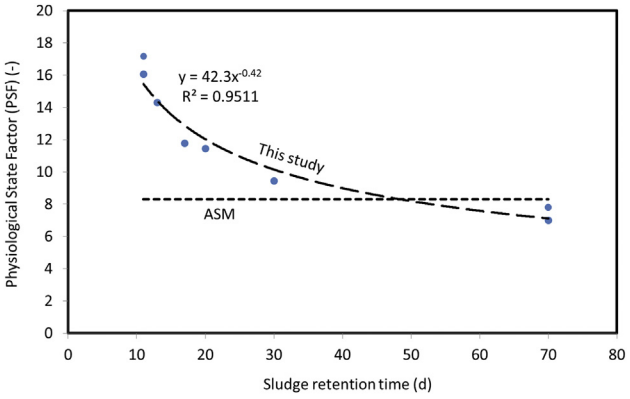

Fig. 7. Correlation of the PSF and SRT.

From the perspective of energy conservation the decay rate as identified in aerobic degradation batch experiments might stand predominantly rather for the maintenance energy demand than predation, cryptic growth and other sources of biomass loss. In fact, in a substrate poor environment where the bacterial cell exhibits a low decay rate, it is energetically an advantage not to carry all the equipment for rapid growth "on board" where it has to be maintained for rapid protein production.

Therefore decay can be regarded as a kinetic property that belongs to a bacterial cell at a certain physiological state. Taking this into consideration, it is not surprising that b_e correlates well with μ_{max}. In fact a similar linear relationship between endogenous respiration in a carbon limited medium was described by Neijssel and Tempest (1976) for pure cultures of Klebsiella aerogenes.

These experimental results are notable because in recent activated sludge modelling neither a variable kinetic approach nor a link between growth and decay rates are recognized. In particular, the model response for sludge production is strongly influenced by the decay rate parameter, where a small decay rate yields a high sludge production. To explain the good predictive capability of the existing models processes like the reduction of "unbiodegradable organics" (X_U) in activated sludge as described by Spérandio et al., 2013, Ramdani et al. (2010), Friedrich et al. (2015) and others have to be taken into account.

However, the variable kinetic approach especially the

variability of the decay rate will lead to a more balanced active biomass fraction for higher SRTs. An activated sludge with an SRT of 70 days (sludge C) will still have an active biomass fraction of more than 35% and will not be abandoned of bacteria as predicted with the constant growth kinetics approach according to the recent ASMs with an active biomass fraction of less than 15%.

3.5. Defining the physiological state of activated sludge samples

From the results of this research it can be summarized that the maximum specific growth rate and the decay rate of X_{OHO} can be regarded as closely related to each other and the ratio of both parameters is indicative for a certain physiological state of the active biomass in activated sludge. Therefore it is useful to define a physiological state factor (PSF) as the ratio of μ_{max} and b_e:

$$PSF = \frac{\mu_{max}}{b_e} \ (-) \tag{10}$$

A high PSF describes a microbial community that is physiologically growth-optimized. A low PSF describes a microbial community that is physiologically survival-optimized. Fig. 7 shows a good correlation of PSF with the SRT based on an exponential function. However, using the ratio of $OUR_{max}(t_D,0)/OUR_e(t_D,0)$ instead of μ_{max}/b_e would create different values for PSF but would result in the same function. Transferring the idea of the physiological state factor to the example of the Herbert model from Eq. (2) as representative for the existent ASMs would yield into a horizontal line with a constant value of PSF = 2.0/0.24 = 8.3.

4. Conclusions

The constant growth kinetics as applied in ASMs for SRTs of 3–20 d does not reflect the reality of microbial life with respect to the physiological adaptation of bacterial cells to nutrient supply. For the identification of growth kinetics that can be reliably applied in ASMs the culture history has to be recognised.

This study confirms that for the determination of the decay rate parameter b_e from the exponential decrease of OUR_e it is necessary to exclude the degradation of rapidly degradable cell constituents. In general the decay rate becomes smaller as in recent ASMs applied.

Furthermore it is notable that by transferring the characteristic of the aerobically degraded sludge (OUR_e, b_e) into an environment of excess substrate supply (OUR_{max}) a mathematical formulation can be introduced to describe the maximum specific growth rate μ_{max} mainly with the ratio OUR_{max}/OUR_e.

Using this approach it becomes possible to identify a physiological adaptation of μ_{max} to starvation within a degradation test. In the low SRT sludge μ_{max} is decreasing rapidly for approximately two weeks but from then on increases significantly. Looking at the activated sludge sample from a high SRT system it becomes evident that the physiological adaptation to starvation already occurs in the environment of the WWTP.

Consequently, if the value for the maximum specific growth rate of bacteria is the result of a physiological adaptation then the Monod-term describing the actual specific growth rate of bacteria is only valid for a certain phenotype and therefore a certain physiological state of the bacterial cell.

Even if growth and decay are antagonistic processes it will be possible to develop a consistent variable kinetic approach for activated sludge that is based on and characterised by the obtained strong correlation of the maximum specific growth rate μ_{max} and the decay rate b_e.

By defining the ratio of μ_{max}/b_e as a physiological state factor (PSF) it can be concluded that a high PSF characterises a microbial system that will be growth-optimized and a low PSF describes a microbial system that will be survival-optimized.

The physiological adaptation of active biomass in activated sludge is of significant influence to the predictive capability of activated sludge models. In particular further characterisation of growth kinetics for extreme environments (SRT <1 d and >50 d) would reveal physiological properties of bacteria in activated sludge that are precious towards an improvement of the experimental basis for a general variable kinetic approach.

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Glossary

b_e: First order rate constant for the decay of ordinary heterotrophic organisms (d^{-1})
b_{max}: First order rate constant for the decrease of OUR_{max} over the degradation time (d^{-1})
μ_{max}: Maximum specific growth rate of ordinary heterotrophic organisms (d^{-1})
f_U: Unbiodegradable fraction of ordinary heterotrophic organisms (–)
K_s: Half-saturation constant of the Monod kinetic term describing the affinity of bacteria to a certain substrate (mg/l)
OUR: Measured endogenous oxygen uptake rate (mg O_2/(l*h))
OUR_e: Endogenous oxygen uptake rate due to the respiration of ordinary heterotrophic organisms (mg O_2/(l*h))
$OUR_e(0)$: Initial endogenous oxygen uptake rate of ordinary heterotrophic organisms in a degradation test (mg O_2/(l*h))
$OUR_e(t_D,0)$: Initial endogenous oxygen uptake rate in a growth test (mg O_2/(l*h))
OUR_{max}: Maximum oxygen uptake rate at substrate saturation (mg O_2/(l*h))
$OUR_{max}(t_D,0)$: Maximum oxygen uptake rate at time t_D of the degradation test and at the time of spiking the sample with acetate (mg O_2/(l*h))
q_{STOR}: First order rate constant for the degradation of stored organic material of ordinary heterotrophic organisms (d^{-1})
SRT: Sludge retention time (d)
t_a: Adaptation time to starvation within the degradation test (d)
t_D: Degradation time within the degradation test (d)
t_G: Growth time within the growth test (h)
S: Substrate concentration (mg/l)
VSS: Volatile suspended solids (mg VSS/l)
X_{OHO}: Concentration of ordinary heterotrophic organisms (mg COD/l)
X_{STOR}: Stored organic material concentration of ordinary heterotrophic organisms that causes an high initial respiration rate at the beginning of degradation test (mg COD/l)
X_U: Unbiodegradable organic material in activated sludge (mg COD/l)
Y: Biomass yield of ordinary heterotrophic organisms (g COD_X/g COD_S)