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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