Michael Friedrich

Adaptation of growth kinetics and degradation potential of organic material in activated sludge

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It is not because things are difficult
that we do not dare,
it is because we do not dare
that they are difficult.

Lucius Annaeus Seneca

to my wife Gitta

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Acknowledgments

A thesis is tremendously time consuming. Without a family accepting and supporting this work one should not even start to write a thesis. I am deeply grateful to my family and in particular to my wife that I could spend so much time for this project.

To work as intensive at a project as I had to it is a prerequisite to develop certain personal qualities. The fundamentals for this process were laid by my parents. They surely had the highest impact by their love, their determination to educate me and by their pure existence throughout my childhood until now.

If there is something like an initialization of scientific interest, I received that at the University of Cape Town during my practical course in the Water Research Group in 1992. It was the generosity of Prof. Marais and Mark Wenzel and all the other group members that implanted in me the joy of thinking into the depth of wastewater related problems.

Starting my professional career I was lucky to meet Ulrich Kotzbauer, who took my shyness away of tackling real engineering problems and who was a solid pillar during starting up my own business. His iron will in facing challenges was always an ideal for me and helped me in proceeding with this work even if sometimes it seemed hopeless.

Knowing Imre Takács already for a long time, he became my scientific supervisor and my confidant. He was an endless source of experience, patience, encouragement and kept me grounded when I thought I was lost. During this work he became a good friend, which for me means even more than solving a big scientific problem.

I am very grateful to John Copp who reviewed Chapter 4 of this thesis very thoroughly thereby improving the chance to get accepted for publication.

I am happy to have multitasking colleges like Britta Schmolinski, who helped me in the laboratory work. I am also glad to have colleges bearing my passion for research and I am grateful for the help they gave by solving all the “ordinary” problems of daily working life.

Last but not least I thank all the water companies that supplied the “material” of interest – the activated sludge I was depending on to execute all the batch tests.

And finally I am deeply grateful to Prof. Jens Tränckner, who was willing to accept and introduce me as a PhD student at the University of Rostock. He spent a lot of time by thoroughly reading, writing comments and questioning what seemed to be clear at first sight, which improved the thesis at vulnerable parts.

List of Abbreviations

ASM	Activated sludge model
ATU	Allylthiourea
b	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})
COD	Chemical oxygen demand
DNA	Deoxyribonucleic acid
DO	Dissolved oxygen
EPS	Extracellular polymeric substances
F/M	Foot to microorganism (mg COD/mg COD)
F_a	Active fraction of organic biomass (mg COD/mg COD)
f_{CV}, i_{CV}	COD content of VSS (mg COD/mg VSS)
f_{DEG}	degradable fraction of organic material (mg COD/mg COD)
f_N	Nitrogen fraction of ordinary heterotrophic organisms (-)
f_p	Endogenous residue fraction within the death regeneration approach (-)
f_{STOR}	fraction of stored substrate that is degraded at the beginning of a degradation experiment (mg COD/mg COD)
f_U	Unbiodegradable fraction of ordinary heterotrophic organisms (mg COD/mg COD)
$f_{U,E}$	Endogenous residue fraction of ordinary heterotrophic organisms (mg COD/mg COD)
$i_{CV,DEG}$	COD content of the degradable VSS (mg COD/mg VSS)
$i_{CV,U}$	COD content of the unbiodegradable VSS (mg COD/mg VSS)
K_s	Half-saturation constant of the Monod kinetic term describing the affinity of bacteria to a certain substrate (mg/l)
MLSS	Mixed liquor suspended solids (mg TSS/L)
n.i.	no information
OHO	Ordinary heterotrophic organisms
OUR	Measured oxygen uptake rate (mg O_2/(l*h))
spOUR	specific oxygen uptake rate (mg O_2/(VSS*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))
P	Period
PE	population equivalents
PHA	Polyhydroxyalkanoate
PSF	Physiological State Factor
R^2	Correlation coefficient
q_{STOR}	First order rate constant for the degradation of stored organic material of ordinary heterotrophic organisms (d^{-1})
q_U	First order rate constant for the degradation of “unbiodegradable” organic material in activated sludge (d^{-1})
SMP	Soluble microbial products
SRT	Sludge retention time (d)
t_a	Adaptation time to starvation within a degradation test (d)
t_D	Degradation time within the degradation test (d)
t_G	Growth time within the growth test (h)
TSS	Total suspended solids (mg TSS/l)
S	Substrate concentration (mg/l)
VSS	Volatile suspended solids (mg VSS/l)
VSS_U	Unbiodegradable volatile suspended solids (mg VSS/l)
WRRF	Water resource recovery facility
X_{DEG}	Degradable fraction of VSS in COD units (mg COD/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)
$X_{U,inf}$	Unbiodegradable particulate organic material in the influent (mg COD/l)
$X_{U,E}$	Endogenous residue of ordinary heterotrophic organisms (mg COD/l)
Y	Biomass yield of ordinary heterotrophic organisms (g COD_X/ g COD_S)
θ	Van’t Hoff-Arrhenius temperature correction factor
μ_{max}	Maximum specific growth rate (d^{-1})

Theses

Bacterial growth kinetics in activated sludge is modelled on the basis of constant mean values for maximum specific growth rates and decay rates. This seems not to reflect the reality of microbial life.

In aerobic degradation batch experiments the exponential decrease of endogenous oxygen uptake rates (OUR_e) is indicative for the kinetics of the decay process. However, experimental evidence shows that the exponential decrease is not ideal. By modelling a first rapid OUR decrease separately from the actual decay process leads to derivation a more adaptive decay rate that shows high values for high loaded and low values for low loaded activated sludge systems.

Therefore the decay rate of active biomass in mixed cultures like activated sludge is not constant as it is believed in recent activated sludge modelling. The decay rate is adaptive to the nutritional state which is given by the systems solids retention time (SRT). This implies that the active biomass fraction is lower in high loaded and higher in low loaded systems as predicted with a constant growth kinetics approach.

The maximum specific growth rate as it can be derived from maximum growth experiments as well as degradation experiments is not constant but yields high values for high loaded systems (low SRT) and low values for systems where starvation dominates (high SRT).

Both the maximum specific growth rate and the decay rate can be correlated to each other with a high correlation coefficient showing that the kinetic properties of a bacterial cell in general are physiologically adaptive to the loading conditions.

Using the ratio of the maximum specific growth rate and decay rate as a physiological state factor describes and quantifies the kinetic properties of active biomass based on their physiology.

The classification of activated sludge into biodegradable and unbiodegradable solids has a long history. However, comparing volatile suspended solids (VSS) and OUR profiles from aerobic degradation batch experiments it can be shown, that “unbiodegradable” solids are subjected to degradation even if the rate is small.

Preface

For a long time writing a PhD-thesis for me was something so big, that I didn't feel having the strength, the stamina and of course the time to cope with such a project. However, scientific work became more and more essential to me even if it never dominated my daily routines. In particular experimental work mainly based on respirometry was useful in finding insights into complex microbial issues even in my profession as a practicing engineer.

As the laboratory equipment improved, the questions that could be asked and answered became more scientific. It was clear to me, that a further increase of quality was only possible by input from outside my environment. I was lucky to win Imre Takács, who is one of the most inspiring and experienced scientists in process engineering, to support me as supervisor and to introduce me to the highest possible scientific level.

At this time I already had executed many aerobic digestions batch experiments as well as activity measurements at substrate saturation. It was just unavoidable to end up dealing with decay of ordinary heterotrophic organisms (OHOs) in terms of a first research project. There were obvious signs of mismatches between the widely used decay models and experimental observations, which were an additional motivation to dig deeper into this issue. To meet the high quality standards of the experimental procedure all the experiments originally carried out with an open surface respirometer were repeated with a new, closed respirometer configuration. Even if this was a lot of work it was of importance to exclude oxygen intrusion via an open water surface and in that way to gain a high confidence in experimental results. The findings of this work were published in Water Research in 2013 (Friedrich and Takács, 2013) and are presented here in Chapter 2.

For me the discovery of consistent variable and therefore adaptive decay kinetics of OHOs was just the fish on the hook. Finding a link of decay to bacterial growth kinetics would mean to bring the fish into the boat. It was just at this point that I decided to carry on with that project and expand it to a PhD project.

Therefore I designed and executed a combination of aerobic degradation experiments together with aerobic growth experiments. The results were surprising

but consistent with the initial assumption that physiological adaption effects not only the decay rate but also the specific maximum growth rate and therefore consistent with the fact that both antagonistic processes can be closely correlated to each other. Within this work for me it was of particular interest to find a way to describe the specific maximum growth rate (μ_{max}) without knowing the concentration of OHOs. The concentration of OHOs in activated sludge can only be obtained by using an activated sludge model (ASM). However, I was suspicious against the existing ASMs, in particular because of their constant growth kinetics. By solving this problem it became apparent that information about microbial activity during starvation, measured as endogenous oxygen uptake rate (OUR_e) as well as conditions of substrate saturation, measured as maximum oxygen uptake rate (OUR_{max}) can be used to calculate μ_{max}. Furthermore the ratio of OUR_{max}/OUR_e is indicative for the physiological state of a microbial population. This work was published in Water Research as well in 2015 (Friedrich et al., 2015) and as Chapter 3 is part of this thesis.

An important implication within the theory of physiological adaptation of bacterial population to starvation is the development of a starvation survival response. In starvation it is useful to find access to substrate sources, which were energetically not of interest in times of excess substrate supply. This could be observed by comparing the OUR and VSS profiles of aerobic degradation batch experiments. It is the theory that endogenous respiration is performed due to the degradation of a degradable fraction of OHOs, which corresponds to the degradable fraction of VSS (X_{DEG}) in starving activated sludge. The OUR after 3 to 4 weeks of degradation time is supposed to be close to zero and the slope of the linearized OUR profile yields the decay rate parameter. However the characteristic decrease of VSS within this time should end at a VSS concentration that represents the unbiodegradable fraction of VSS (X_U). However, the experimental results did not show a flat line for the residual X_U concentration. Therefore it was concluded that the biodegradable fraction had to be expanded at the expense of the unbiodegradable fraction of VSS. In chapter 4 of this thesis, which is published in Water Environment Research (Friedrich et al., 2016), a procedure is developed to quantify X_U in the course of a degradation experiment. From the X_U profile the degradation rate is evaluated. It should be noted, that this part of the project is a by-product and arose from the mismatch of OUR and

VSS based decay rate determination. However this work is of high importance for activated sludge modelling as well as for understanding physiological adaptation of OHOs.

Although all three parts of this thesis could stand in themselves, they document the process of evolution of knowledge within this project about growth kinetics of OHOs. This process is based on questioning the existing models and is substantially based on experimental work and theoretical deduction.

The first chapter of this thesis introduces my personal doubts about the existing decay model. To relate these doubts to the fundamental knowledge about microbial life the adaptability of bacteria to environmental exigencies is discussed. Finally, due to the immense importance of the decay process for microbial growth kinetics and therefore for activated sludge modelling historical milestones in this field of research are presented.

Furthermore, additional chapters were inserted into the thesis because they address important experimental and theoretical issues I had to deal with, but could not include into the publications.

Chapter 2.5.1 describes problems using allylthiourea (ATU) for the suppression of nitrification and Chapter 2.5.2 deals with the systematic errors generated by open surface respirometers. However, Chapter 3.7, in my opinion, is one of the most interesting chapters of this thesis, asking for the experimental identifiability of the specific maximum growth rate of OHO in activated sludge. Does bacteria in activated sludge grow primarily in size or in number? This question is fundamental to growth kinetics and the experimental results of this work might help to find an answer and are therefore worth to be included in this thesis.

1. Introduction

1.1 Initial doubts

Initially, without recognizing and knowing the rich literature about decay of OHOs in the activated sludge process of the past 50 years for me it was the way of modelling decay as a first order reaction which was puzzling.

The rate of a first order reaction is only dependent on the constituent that is changed by the reaction. The rate constant describes how many percent of the constituent is changed per time unit with respect to the momentary availability of the constituent. It was this first order reaction kinetics which was found to be best suitable describing the kinetics of the decay process in activated sludge (Herbert, 1958). Thereby the reduction rate of OHOs depends only on the presence of OHOs in the system. And consequently OHOs and possibly bacteria in general die at the same rate. Can this model approach reflect reality?

To understand the simplicity of this idea it is helpful to take instead of a chemical reaction an example from hydrodynamics like the emptying of a water vessel by an outlet at the bottom (see Figure 1.1).

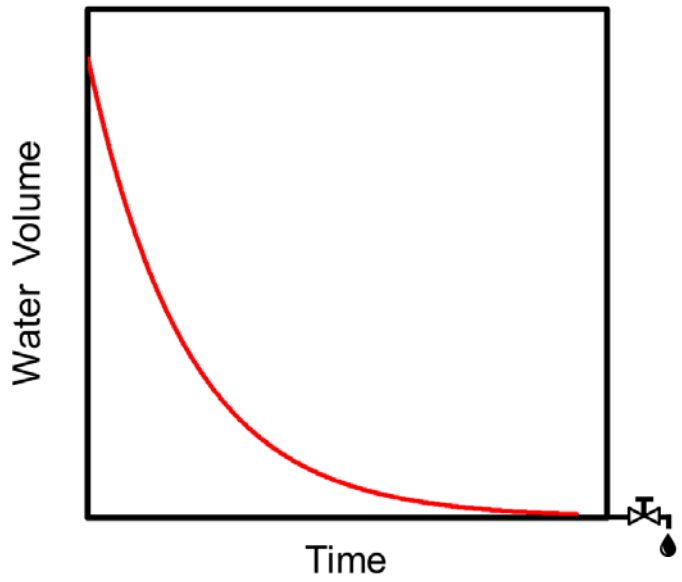

Figure 1.1: First order reaction kinetics of emptying a water vessel

The rate at which the water volume (l/s) runs out of the outlet decreases with decreasing water volume, but the rate parameter k describing the process rate of emptying by Eq. 1.1 is a constant.

$$\frac{dV_{Water}}{dt} = -k \cdot V_{Water} \qquad \text{Eq. 1.1}$$

Transferring this reaction kinetics from water to a bacterial cell of an OHO, it is hard to imagine that the process velocity of the reduction of the bacterial cell mass should follow the same basic assumption as the emptying of a water vessel.

First, it is the **nature** of the constituent that is changed. If water can be regarded as a homogenous fluid, which won't change its chemical and physical characteristic in the vessel and the vessel as an ideal system boundary a bacterial cell in structure and composition is incomparably more complex.

Second, the **driving force** that makes the water ran out of the vessel is the gravity and in particular the hydrostatic pressure at the outlet. The driving force for the bacterial cell to metabolise the own cell mass is the lack of external substrate. Following the first order reaction kinetics for the decay process without external substrate a bacterial cell would use internal substrate at the same rate from the beginning of starvation until all the internal substrate is degraded. In other words the driving force would empty the bacterial cell of internal substrate in the same way as the vessel rans out of water and inevitably the bacterial cell would die defenceless and with a constant rate.

Third, if it is of advantage to keep the water in the vessel as long as possible, a **manipulation** on the tap at the outlet would reduce the rate parameter of the first order reaction. If survival of the bacterial cell can be improved for instance by reducing the internal energy requirements just to maintain basic cell functions, it is quite certain that in the evolution of prokaryotes one or more of these regulatory mechanisms were developed.

Using this comparison it was my initial impulse to question that kinetically it is the same to empty a water vessel and a bacterial cell. Nevertheless, it will be shown, that a first order reaction kinetics is a good approximation to describe endogenous

processes, even if the nature of degraded cell material is far away from being homogenous. Furthermore it will be shown that the driving force (lack of external substrate) will not only induce degradation of cell internal material but will stimulate an adjustment of the cell internal macromolecular composition of OHOs to synchronize the energy requirement of the cell with the availability of an external energy source.

1.2 Adaptability of bacteria to environmental exigencies

The evolutionary history of prokaryotic bacteria is as long as 3.5 billion years. In contrast eukaryotic life appeared one billion years later on earth (Futuyma and Douglas, 2005). Both concepts differ significantly from each other. While bacteria express their diversity and survival in terms of physiology and metabolism the success of eukaryotes is based on the development and adaptation of structures and behaviour (Nealson and Stahl, 1997).

Compared to eukaryotic cells (20 µm to several mm) the range of bacterial cell size is restricted to 1 – 10 µm (White, 2007). The resulting surface to volume value for bacteria is 100 to 1000 times higher than for eukaryotic cells, which makes it difficult for eukaryotes to energetically compete. Taking this into account the contribution of higher organisms to the degradation of organic compounds in activated sludge might be rather limited.

However, bacteria have to transport substrate through a rigid cell wall and are not in the possession of structural or behavioural means as eukaryotes to capture substrate by phagocytosis or food engulfment (Nealson and Stahl, 1997). But they developed a highly adaptive strategy of food preparation and uptake into the bacterial cell. In the presence of large polymeric molecules the bacterial cell produces substrate specific extracellular enzymes to convert the polymers into small oligomers and monomers. For the uptake of substrate bacteria developed a substrate specific transport system to move the prepared pieces of nutrients against concentration gradients into the cytoplasm.

But more importantly the metabolic versatility of bacteria exceeds that of eukaryotes by far. Eukaryotes can only use a limited number of organic substrates as energy source and only oxygen as oxidant, while bacteria can make use of a wide range of energy sources and oxidants in a way that they can adapt to and be found in nearly every imaginable environment. Due to this distinct metabolic versatility bacteria could develop various responses to the environmental stress as survival strategies.

The most prominent adaptation strategy is the starvation-survival response (Morita, 1993) as it is expected to be found in high SRT activated sludge systems. Starving bacterial cells alter their macromolecular composition. Daigger and Grady (1982) named this change physiological adaptation and the result of this adaptation is referred to as the physiological state of the bacterial cell.

The starvation-survival response generates a low physiological state where the macromolecular composition of a bacterial cell is altered and reduced to the required minimum. Concomitantly the cell size is reduced to less than 1.0 µm (White, 2007) probably even less than 0.5 µm (Morita, 1993). The lipid as well as the phospholipid content of the bacterial cell decreases to less than 1% after a period of 7 days of starvation (Hood et al., 1986). This indicates a reduction of the cell membrane since the phospholipids are mainly found in this part of the cell. In addition, it was found that after 30 days of starvation bacterial cell membranes where extremely difficult to rupture. It was assumed that starved bacteria alter the cell surface to become resistant to degradation as it is known for spore-forming bacteria (Morita, 1993).

Another important regulation of the starvation-survival responds is the reduction of the protein synthesizing system, the ribosomes. This process is known as ribophagy and even if it is not fully understood, it is clear that the cell profits energetically from the degradation of ribosomes (White (2007); Cebollero et al. (2012); MacIntosh et al. 2011)).

Obviously, in an aerobic environment the reduction of the cell membrane and the reduction of the number of ribosomes contribute significantly to the endogenous respiration in activated sludge. However, with respect to growth kinetics it is easy to understand that a fully equipped mature bacterial cell has the potential to grow faster

than a starving cell that has to build up the metabolic machinery as soon as the supply of nutrition improves.

Furthermore, Kurland and Mikkola (1993) investigated the impact of the nutritional state on the microevolution of ribosomes and found that bacteria at a high nutritional state develop a different phenotype than bacteria which have to adapt to starvation. They observed bacteria which were grown in the presence of excess substrate and found that by sudden withdrawal of substrate these bacteria died faster than bacteria which were grown under substrate limitation and therefore adapted to starvation. Interestingly it was found for natural isolates that growth rates correlate well with death rates at a correlation coefficient of 0.84. Based on these results it was concluded that the slower the growth rate and the slower the translation efficiency of ribosomes, the more resistant the bacteria are to the exigencies of starvation.

1.3 Modelling decay of ordinary heterotrophic biomass

Among the first researchers who recognised decay as an important process, that had to be considered in ASM, were Herbert (1958) and Pirt (1965). They tried to explain a reduced biomass yield, observed at higher SRT. Herbert (1958) suggested that beside an anabolic metabolism there must be an endogenous metabolism exclusively performed to maintain the basic cell function. To model this process he introduced a first order reaction kinetics. However, Herbert suggested that this endogenous process runs independently of the “normal” growth processes as described by Monod (1949).

Pirt (1965) on the other hand thought that the uptake of external substrate is primarily due to the maintenance energy requirements and anabolism is just the result of the degradation of surplus substrate.

However it was Herbert’s approach that became widely used in ASM because it could explain better the production of an endogenous residue that is produced in the consequence of endogenous degradation of cell material.

The first comprehensive dynamic ASM was the result of extensive research by the Water Research Group at the University of Cape Town in South Africa. This group

was led by Prof. G. v. R. Marais. Using respirometry Marais et al. (1976) suggested that the decay rate of heterotrophic organisms corresponds to the endogenous respiration rate in an aerobic digestion batch experiment (see Figure 1.2 (A)). The decay rate is obtained by plotting the OUR values on a logarithmic scale over the digestion time, where the slope of that line is the decay rate parameter.

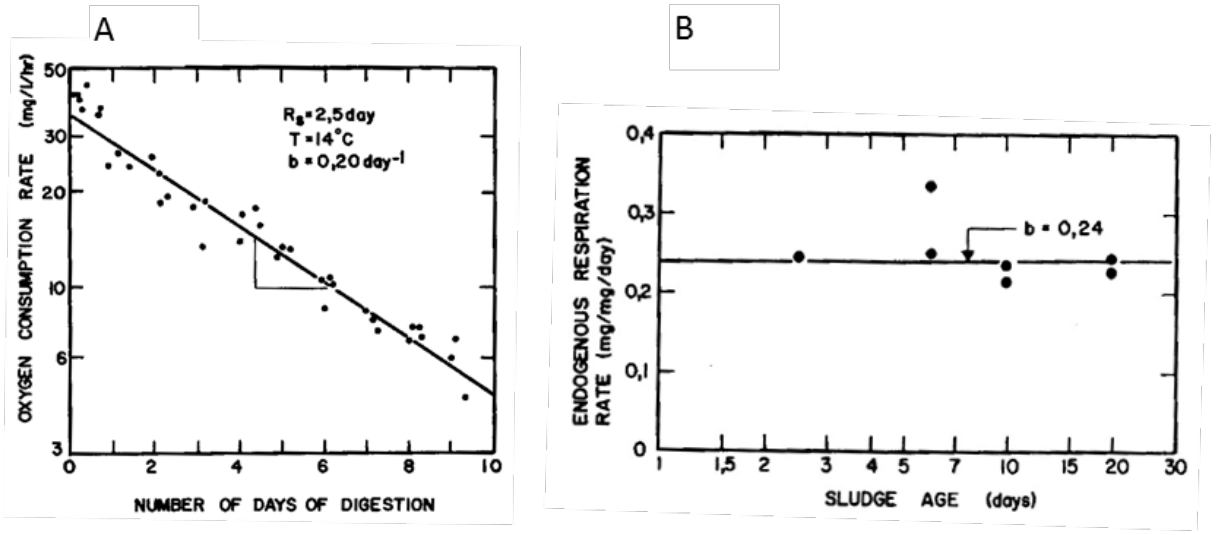

Figure 1.2: (A) Determination of the decay rate by respirometry and (B) constancy of decay rate with sludge age from Marais et al. (1976)

However, Marais et al. (1976) presented with Figure 1.2 (B) experimental results showing the constancy of the decay rate with respect to SRT. The mean value for the decay rate of OHO was found to be 0.24 d^{-1}. In the following decades very few attempts were made to question this constancy theoretically and experimentally, although Lee et al. (2003) found significant differences in the heterotrophic decay rate for SRTs of 10 und 20 d. Moreover, research was published recently confirming the constancy of heterotrophic decay rates (Ramdani et al., 2010) for SRTs of 5 and 10 d as well as the value of the decay rate parameter as determined by Marais et al. (1976).

A further milestone in modelling decay of OHOs was the introduction of the death-regeneration-approach by Dold et al. (1986). At this time there was no microbial explanation and therefore no modelling concept available for the observation that activated sludge after a period of anaerobic conditions showed an increased OUR, indicating a substrate accumulation. Furthermore it was thought that the decay

process is not influenced by the presence or absence of an electron donor. Dold et al. (1986) hypothesised that if the decay under anaerobic conditions doesn't stop the decayed material will accumulate and will be oxidized in the subsequent aerobic or anoxic environment, which creates a death regeneration cycle as displayed in Figure 1.3 in ASM1 (Henze et al., 1987). However, the decay rate still measured as the net decrease of endogenous respiration had to be transferred into the death regeneration model by the following relationship:

$$b = \frac{b'}{(1 - Y \cdot (1 - f_p)} \qquad \text{Eq. 1.2}$$

Where:
- b' decay rate measured as decrease of endogenous respiration
- b decay rate within the death regeneration approach
- Y heterotrophic biomass yield (Y = 0.67 mg COD_X/COD_S)
- f_p endogenous residue fraction within the death regeneration approach (f_p = 0.08)

If the decay rate is measured as the decrease of endogenous respiration of b` = 0.24 d^{-1} the corresponding decay rate of the death regeneration approach is b = 0.62 d^{-1}. Reviewing the literature it was confusing to deal with decay rates if the respective model was not properly explained.

However, the decay process of ASM1 (Henze et al., 1987) and ASM2 (Henze et al., 1995), two widely used ASMs, are based on the death regeneration approach.

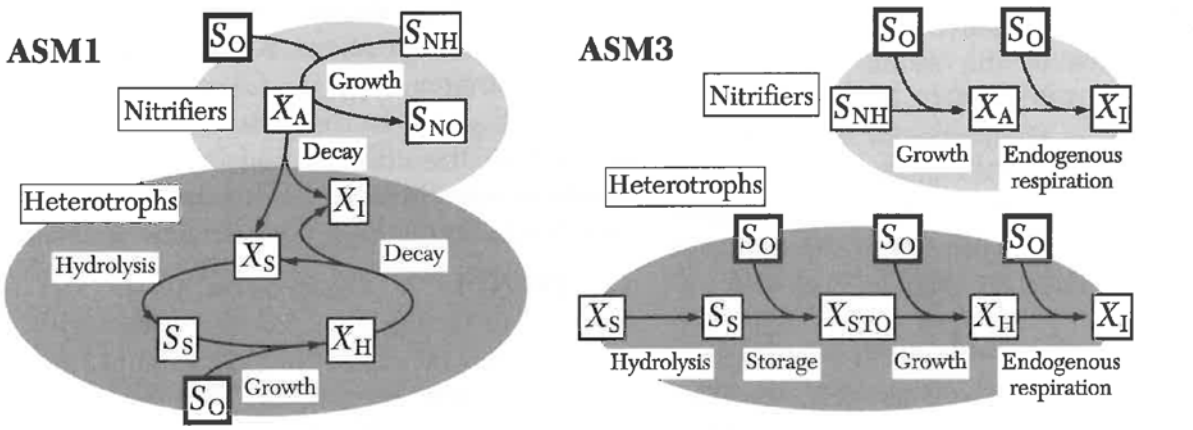

Figure 1.3: Flow of COD in ASM1 and ASM3 in Gujer et al. (1999)

In ASM3 (Gujer et al., 1999) the decay process of OHOs is modelled as endogenous respiration. The substrate accumulation is explained by an electron donor independent hydrolysis process that makes the substrate available even under anaerobic conditions and oxidises it under aerobic or anoxic conditions.

2. A new interpretation of endogenous respiration profiles for the evaluation of the endogenous decay rate of heterotrophic biomass in activated sludge

2.1 Introduction

One of the main objectives of activated sludge modelling is the prediction of sludge production in WRRFs. On the one hand, sludge production is the result of biomass growth and unbiodegradable material accumulation. On the other hand, particularly in systems with a longer solid retention time (SRT), growth antagonistic processes play an important role. These processes are generally described as lysis or decay of active biomass and slow degradation of other organic material (Henze et al. 2000).

Experimentally it is common practise to use aerobic batch digestion experiments to gain information about the processes that are involved in degradation of activated sludge (Spanjers et al. 1995, 1996). The assumption is that this information is representative to describe decay in an environment where decay and growth takes place simultaneously. The metabolic explanation of these processes is expressed in concepts like endogenous respiration (Gujer et al. 1999), death-regeneration (Dold et al. 1980), maintenance (van Loosdrecht et al. 1999) or predation (Moussa et al. 2005). All these concepts are based on the assumption that organic material (X_{ORG}) in activated sludge consists of a biodegradable fraction ($X_{ORG,DEG}$) and an unbiodegradable fraction ($X_{ORG,U}$).

$$X_{ORG} = X_{ORG,DEG} + X_{ORG,U} \quad \text{(mg COD/l)} \qquad \text{Eq. 2.1}$$

To convert organic material that is measured as volatile suspended solids (VSS) into chemical oxygen demand (COD) units typically a constant factor f_{CV} = 1.42 g COD/g VSS is used (Henze et al. 2000).

The biodegradable fraction (f_{DEG}) of X_{ORG} in long SRT systems consists overwhelmingly of ordinary heterotrophic organisms (X_{OHO}), whereby for reasons of simplicity the very small fraction of autotrophic active biomass is included in X_{OHO}. However, X_{OHO} has an unbiodegradable fraction f_U that is called endogenous residue (f_U*X_{OHO}) and is a left over from the degradation of active biomass X_{OHO}.

$$X_{ORG,DEG} = X_{ORG} \cdot f_{DEG} = (1 - f_U) \cdot X_{OHO} \quad \text{(mg COD/l)} \qquad \text{Eq. 2.2}$$

The pool of unbiodegradable organic material ($X_{ORG,U}$) is fed by unbiodegradable organic compounds from the influent ($X_{ORG,U,inf}$) and by X_{OHO} in terms of endogenous residue.

$$X_{ORG,U} = X_{ORG,U,inf} + f_U \cdot X_{OHO} \quad \text{(mg COD/l)} \quad \text{Eq. 2.3}$$

In the endogenous respiration concept, the biodegradable fraction of X_{OHO} is regarded as a homogenous substrate that undergoes self-destruction in the absence of external substrate. Here the meaning of homogenous refers to composition (COD, nitrogen, phosphorus) and biodegradability. The degradation process of X_{OHO} is modelled with Eq. 2.4 and Eq. 2.5, where the rate constant of degradation is generally called the decay rate parameter (b). In activated sludge models, the decay rate parameter is assumed to be constant.

$$\frac{dX_{OHO}}{dt} = -b_{OHO} \cdot X_{OHO} \quad \text{Eq. 2.4}$$

$$X_{OHO(t)} = X_{OHO(0)} \cdot e^{-b_{OHO} \cdot t} \quad \text{(mg COD/l)} \quad \text{Eq. 2.5}$$

The concentration of X_{ORG} at any time within a degradation experiment can be expressed with Eq. 2.6 and is referred as the VSS based method to determine the decay rate b_{OHO} (Ramdani et al. 2010):

$$X_{ORG(t)} = X_{ORG,U,inf} + f_U \cdot X_{OHO} + (1 - f_U) \cdot X_{OHO(0)} \cdot e^{-b_{OHO} \cdot t} \quad \text{(mg COD/l)} \quad \text{Eq. 2.6}$$

The endogenous respiration is limited by the internal carbon source that is $(1\text{-}f_U)*X_{OHO}$. The endogenous respiration rate $OUR_{OHO}(t)$ during the aerobic degradation experiment is modelled with Eq. 2.7 and Eq. 2.8 and refers to the OUR based method to determine the decay rate b_{OHO} (van Haandel et al. 1998, Ramdani et al. 2010):

$$OUR_{OHO}(t) = (1 - f_U) \cdot \frac{X_{OHO}}{dt} \quad \text{Eq. 2.7}$$

$$OUR_{OHO}(t) = (1 - f_U) \cdot X_{OHO(0)} \cdot b_{OHO} \cdot e^{-b_{OHO} \cdot t} \quad \text{(mg } O_2\text{/(l*h))} \quad \text{Eq. 2.8}$$

Solving Eq. 2.8 for t = 0 yields:

$$OUR_{OHO}(0) = (1 - f_U) \cdot X_{OHO}(0) \cdot b_{OHO} \quad \text{(mg } O_2\text{/(l*h))} \quad \text{Eq. 2.9}$$

Rearranging Eq. 2.9 for b_{OHO} shows, that a constant decay rate implies a constant ratio of $OUR_{OHO}(0)$ to $X_{OHO}(0)$.

$$\frac{OUR_{OHO}(0)}{(1 - f_U) \cdot X_{OHO}(0)} = b_{OHO} \quad (h^{-1}) \quad \text{Eq. 2.10}$$

It is important to note that in this model the degradation characteristics of X_{OHO} are independent of the loading of external substrate in the WRRF. Therefore the endogenous decay rate parameter is independent of the F/M ratio and independent of the SRT.

Experimental evidence for the validity of the endogenous respiration model comes from Marais et al. (1976). Using the OUR method, the value of b_{OHO} was found to be 0.24 d^{-1} and independent from SRT in the range of 2.5 to 30 days. Similar results were obtained by van Haandel et al. (1998), who extended the measured parameters by the degradable VSS, the formed nitrate and the loss of alkalinity. In a more recent work of Ramdani et al. (2010), activated sludge was fed with acetate and produced a decay rate of 0.23 d^{-1} for a SRT of 5 and 10 days for both the VSS and the OUR method.

However, from the literature review that is summarized in Table 2.1 it can be stated that:

The reported **endogenous decay rates** b_{OHO} vary significantly between values of 0.059 d^{-1} and 0.500 d^{-1}.

Data for heterotrophic decay rates were reported for four **methods**. The largest number of reported decay rates are based on the above described OUR and VSS based method. There are a few references that show decay rates which were determined with the OUR_{max} method, where not the endogenous respiration but the respiration at substrate saturation was measured and linearized over the time of an aerobic digestion batch experiment.

Table 2.1: Literature review of decay rates and additional information of determination

Author	Method	b_{20}	R^2	Temp.	T corr.	SRT	Test-Time	Surface	Origin WW	ATU	COD-Bal.
		1/d	-	°C	-	d	d	-		mg/l	%
DWA A131 (2000)	n.i.	0.241	n.i.	n.i.	1.072	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
Gujer et al.(1999)	n.i.	0.200	n.i.	n.i.	1.072	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
Henze et al. (1987)	n.i.	0.240	n.i.	n.i.	1.121	n.i.	n.i.	n.i.	n.i.	20	n.i.
Henze et al. (1995)	n.i.	0.155	n.i.	n.i.	1.072	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
Metcalf and Eddy (2004)	n.i.	0.100	n.i.	n.i.	1.023	n.i.	n.i.	n.i.	n.i.	n.i.	n.i.
Benedek et al. (1972)	OUR_e	0.112	n.i.	20	1.072	n.i.	n.i.	open	municipal	no	n.i.
Lee et al. (2003)	OUR_e	0.240	0.987	20	n.i.	20	10	open	municipal	no	n.i.
Lee et al. (2003)	OUR_e	0.310	0.987	20	n.i.	10	10	open	municipal	no	n.i.
Marais et al. (1976)	OUR_e	0.240	n.i.	14	1.029	2-25	10	open	municipal	n.i.	n.i.
Ramdani et al. (2010)	OUR_e	0.233	0.990	20-24	1.029	5-10	21	closed	synthetic	no	97-103
Siegrist et al.. (1999)	OUR_e	0.500	n.i.	10	1.070	16	7	open	municipal	n.i.	n.i.
van Haandel et al.(1998)	OUR_e. VSS. NO3. Alk.	0.246	0.970	20	1.040	3-10	6	open	municipal	n.i.	n.i.
Adams et al. (1974)	VSS	0.325	n.i.	n.i.	n.i.	n.i.	17	n.i.	n.i.	n.i.	n.i.
Ramdani et al. (2010)	VSS	0.238	0.990	20-26	1.029	5-10	21	closed	synthetic	no	n.i.
Avcioglu et al. (1998)	OUR_{max}	0.075	0.965	18	n.i.	15	n.i.	open	industrial	yes	n.i.
Hao et al. (2009)	OUR_{max}	0.148	0.990	25	1.072	10	7	closed	synthetic	5	n.i.
Lavallée et al. (2002)	OUR_{max}	0.400	>0.900	20	n.i.	5-7	21	closed	municipal	25	n.i.
Brands et al. (1994)	DNA	<0.100	>0.980	20	n.i.	low+high	21	closed	n.i.	n.i.	n.i.
Liebeskind et al. (1996)	DNA	0.136	0.950	20	n.i.	2	10	closed	municipal	n.i.	n.i.
Liebeskind et al. (1996)	DNA	0.099	0.960	20	n.i.	6	10	closed	municipal	n.i.	n.i.
Liebeskind et al. (1996)	DNA	0.080	0.960	20	n.i.	9	10	closed	municipal	n.i.	n.i.
Liebeskind et al. (1996)	DNA	0.059	0.890	20	n.i.	24	10	closed	municipal	n.i.	n.i.

n.i. = no information

Two references used the DNA concentration instead of respiration as a parameter to correlate the active biomass concentration in the liquid. The method used in the respective experiment appears to have an influence on the decay rate. In general the OUR and the VSS based methods yield higher decay rates than using the DNA concentration or the OUR_{max} at substrate saturation to describe the decay process. This observation indicates that there might be a difference in endogenous degradation of VSS and in the reduction of activity or viability of active biomass.

The **quality** of the degradation experiments are mostly expressed in terms of R^2 with respect to the linearized data. Thereby it is common practice to regard a reaction with data of $R^2 > 0.95$ as a first order reaction and the substrate that is used up as homogenous. In reality the data can reveal even at higher R^2 a series of different degradation characteristics. However, crucially only a small number of references published the results of the COD balance over the experiment although this is a very important method to show the reliability of the data. Especially the use of open respirometers results in the intrusion of oxygen via the open liquid surface and will affect the COD balance and the decay rate parameter.

There is very little information in the literature about the **initial characteristics of** the used **activated sludge**. From Eq. 2.10 it is evident that OUR(0) and VSS(0) associated with the degradable fraction of VSS is a very important additional information to explain the measured decay rate.

There are no specific studies available that describe the **influence of allylthiourea (ATU)** on the heterotrophic metabolism. Therefore it is possible that inhibition of heterotrophic activity occurs to a certain degree in addition to that of nitrification. For instance the work of Lavallée et al. (2002) has to be evaluated in relation to this specific background.

The data for the **temperature dependency** of the aerobic degradation process expressed as Van't Hoff-Arrhenius temperature correction factor (θ) range from a very low value of $\theta = 1.029$ in Marais et al. (1976) and Ramdani et al. (2010) to very high values of $\theta = 1.121$ in Henze et al. (1987). Therefore it is difficult to compare decay rates that were determined at different temperatures.

There are two references that show a direct dependency of the decay rate on the **sludge age.** Lee et al. (2003) used the OUR method for activated sludge with 10 d and 20 d sludge age and found values for b_{OHO} of 0.24 d^{-1} and 0.31 d^{-1} respectively. Liebeskind et al. (1996) using the DNA method tested activated sludge of four different sludge ages from 2 d to 24 d and observed decay rates from 0.136 d^{-1} to 0.059 d^{-1}.

Taking the above aspects into account it is the focus of this study to find an answer to the following questions:

1. Is the substrate that is used up in aerobic digestion batch experiments as homogenous as initially suggested?
2. Do the active organisms lack the ability of adaptation during the course of the experiment?
3. Is the heterotrophic decay rate constant and independent from the sludge age of the activated sludge in the WRRF?

2.2 Materials and Methods

2.2.1 Method used

In this investigation the OUR based method was used. Experimental details are best explained in Ramdani et al. (2010). It was decided not to run the VSS based method in parallel because the data density of OUR values is much higher and allows more insight into the particular degradation processes. Throughout the experiments no nitrification inhibitor was used.

2.2.2 Origin and characterisation of activated sludge

The activated sludge for the aerobic digestion batch tests was drawn from the return sludge of seven different full scale activated sludge WRRFs ranging from 15.000 to

400.000 PE. With the exception of plant G all WRRFs are equipped with a primary settler. The characteristics are summarized in Table 2.2.

Table 2.2: Characteristics of activated sludge used in aerobic digestion batch experiments

Nr.	Origin	TSS	VSS	VSS/TSS	OUR(0)	spOUR(0)
		g/l	g/l	%	mg O_2/(l*h)	mg O_2/(gVSS*h)
1	A	3.16	2.32	73	13.9	6.0
2	B	3.03	2.24	74	12.3	5.5
3	C	2.96	2.10	71	18.5	8.8
4	A	3.02	2.08	69	10.3	5.0
5	D	3.12	2.30	74	20.6	9.0
6	D	3.44	1.98	70	15.3	6.7
7	E	3.05	2.28	75	16.9	7.4
8	A	2.95	2.07	70	10.2	4.9
9	A	4.31	2.95	69	13.8	4.7
10	F	4.13	3.02	73	20.3	6.7
11	A	3.65	2.55	68	14.7	6.0
12	G	3.94	3.04	77	14.5	4.8

The time from sampling to the start of the experiment was not longer than 1.5 hours. The sludge was diluted with effluent and the temperature adjusted to 20°C. The SRT was taken from operator reports and partially from own investigations. However, the determination of the SRT relies often on assumptions, like the TSS in the waste activated sludge (WAS) and not on measurements. Because of this uncertainty in this study the initial specific OUR(0) (spOUR(0)) in mg O_2/(g VSS*d) of the activated sludge sample is regarded more suitable for describing the loading state of the particular activated sludge. In this context a high F/M ratio (low sludge age) corresponds to a high spOUR(0).

2.2.3 Experimental setup

The respirometer consists of a reactor, an oxygen measuring device (WTW 320i), an aquarium aerator (compressor and fine bubble aerator stone) and the software based on the Labview software package. The compressor was switched on if DO dropped

below a chosen low limit and was switched off if DO exceeded a definable upper limit. The oxygen limits in the batch test where 4 mg O_2/l (lower limit) and 6 mg O_2/l (upper limit). The slope (time from the upper to the lower limit) was computed as OUR in mg O_2/(l*h).

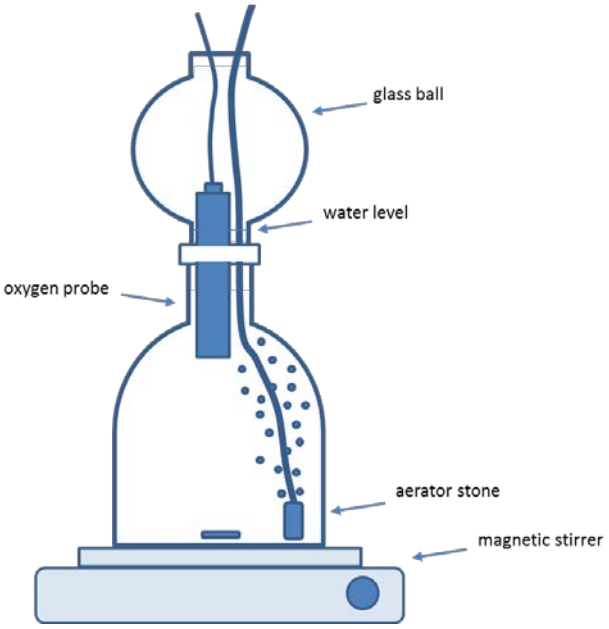

Figure 2.1: Reactor configuration of the respirometer

The reactor configuration is shown in Figure 2.1. It consists of a glass flask of 2.5 l volume and a glass ball on top of that flask to take up the expanded sludge volume during the aeration periods. This arrangement minimizes DO diffusion through the open surface during the DO decline period as the space between the oxygen probe and the aeration tube is very small. Oxygen intrusion was measured by filling the reactor with tap water and adding a small amount of Na_2SO_3 to reduce the DO to zero. The tap water was aerated until the DO started to rise above 0 mg/l and the aerator was switched off. The oxygen intrusion was measured at a certain rotation speed of the mixer as DO increase over the time and was less than 0.01 mgO_2/(l*h). With this reactor configuration it is possible to measure OUR values of less than 0.2 mg O_2/(l*h) with a high degree of linearity of the decreasing DO.

The temperature was controlled via room temperature and varied between 20°C and 22°C. However all OUR values were corrected with a Van't Hoff-Arrhenius

temperature correction $b_T = b_{20}*\theta^{(T-20)}$, with $\theta = 1.072$, following the majority of the authors from the literature review.

The pH was controlled manually by adding NaOH to prevent a pH decrease below 7.0.

2.2.4 Analytical methods

The TSS was measured with 100 ml test volume and an ash free filter. To determine the TSS the sludge was filtered and dried in a microwave (DWA 2003) for 8 min at 700 W. To determine the VSS the dried filter with sample was burned in rapid incinerator SVR/E (Harry Gestigkeit GmbH) at 550°C for 2 h.

To account for the nitrogen, that was released and nitrified, nitrate was measured at the end of the experiment. This was done using a quick test kit from Hach-Lange LCK 340.

2.2.5 COD balance

To check the quality of the experiment a COD balance was performed using the following relationship:

$$\text{COD-Balance} = COD_{start} - (COD_{end} + COD_{loss} + \text{Integral } OUR_e + (NO_3\text{-}N_{end} - NO_3\text{-}N_{start})*4.57) \quad \text{Eq. 2.11}$$

The COD loss is obtained by collecting the TSS that was sticking at the sidewalls of the glass ball on top of the reactor. In average this loss was 2% of the initial TSS. The nitrate in the sample at the beginning of the experiment was always zero due to anoxic sample transportation.

Eight out of these twelve tests had a duplicate test with high reproducibility and good COD balances. Most of them were alike and lead to the same component portions

and rates. However, since the COD balance of all tests was close 100% it was not thought to be necessary to display and discuss duplicate respirograms.

2.3 Results and discussion

2.3.1 Structural analysis of the respirograms

The OUR profile of all respirograms as summarized in Figure 2.2 were analysed. Figure 2.3 shows an example for a typical respirogram in more detail. 3 different phases could be distinguished: Phase 1 was characterized by a rapid OUR decrease that lasts for 0.5 to 4.0 days. It was directly followed by Phase 2 that shows for a couple of days a true exponential decrease of the endogenous respiration rate. This rate was found to be much lower than the OUR decrease of phase 1. Phase 3 was regarded to be the remaining part of the respirograms. In general the OUR of this phase was even slower as in phase 2 and had sometimes but not always increasing and more rapid decreasing sections.

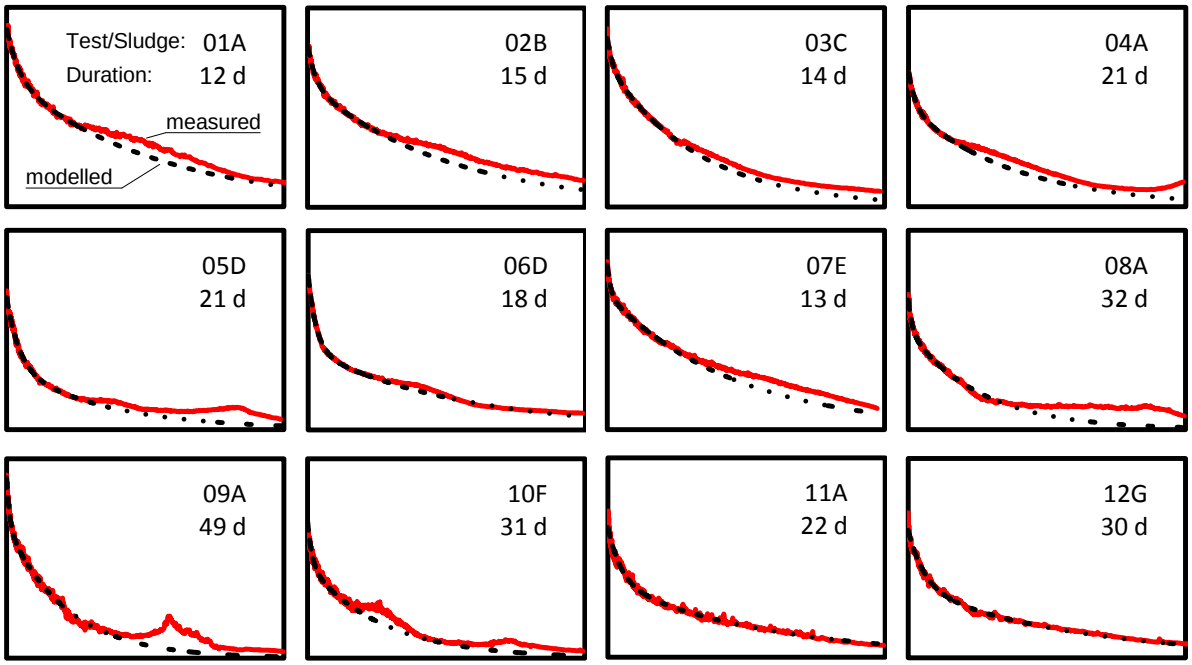

Figure 2.2: Respirograms of aerobic digestion batch tests; x-axis time of experiment; y-axis OUR in mg O_2/(l*h); Reference of respirograms key parameter in accordance to Table 2.2

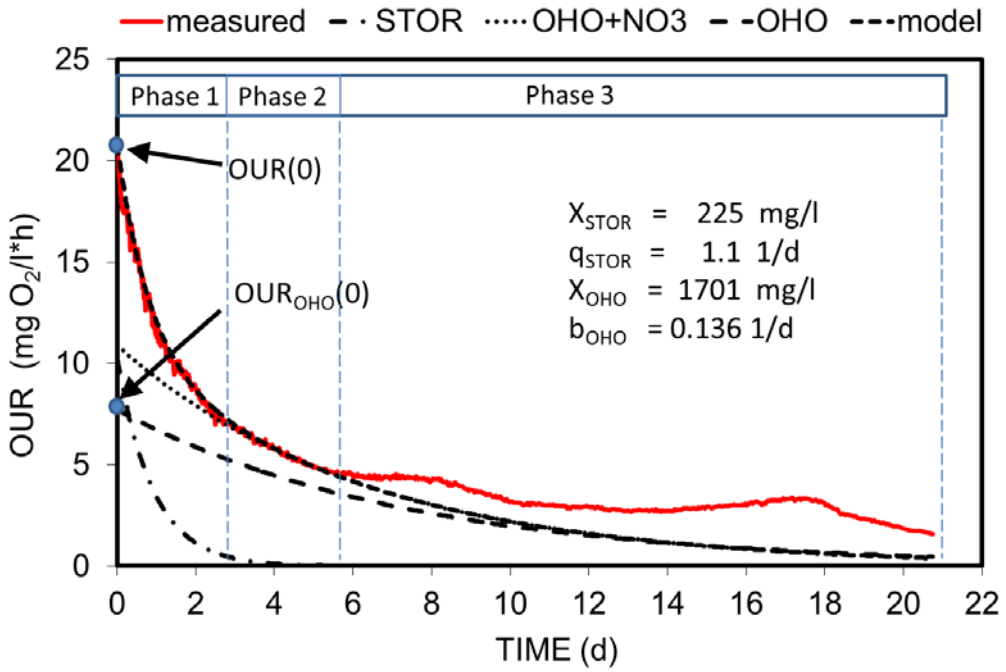

Figure 2.3: Respirogram 05D

From the above analysis it became obvious that for the OUR of phases 1 and 2 a distinct reaction rate was detectable. However the reduced decrease and even the increase of OUR in phase 3 could not be related to the characteristics of phase 1 or phase 2. It is likely that adaptation of active biomass to starvation and the temporary proliferation of higher organisms (predators) lead to deviations from the model of phase 2. However, experiments 11 and 12 did not show these disturbances and phase 2 lasted to the end of the experiment.

2.3.2 Modelling OUR

With regard to phase 1 there are two options to explain the high initial OUR. For option 1 it was hypothesised that within the first rapid reaction storage products (X_{STOR}) e.g. PHA's could have been used up. The theory is supported by the fact that the highest amount of stored and aerobically degraded substrate was found in the sludge of the only WRRF that was run successfully with excess biological phosphorus removal. As option 2 it can also be hypothesised that biodegradable particulate material ($X_{B,inf}$) from the influent is responsible for the high initial OUR. However, in this case a direct correlation of OUR of phase 1 and the spOUR(0),

indicative of loading conditions, would have been observed. This could not be verified. Therefore option 1 was preferred to explain the OUR of phase 1. The $OUR_{STOR}(t)$ was modelled with a first order reaction kinetic according to Eq. 2.12:

$$OUR_{STOR}(t) = q_{STOR} \cdot X_{STOR}(0) \cdot e^{-q_{STOR} \cdot t} \quad (mg\ O_2/(l*h)) \qquad \text{Eq. 2.12}$$

The second, slower reaction of phase 2 was assumed to be due to the degradation of heterotrophic biomass (X_{OHO}) and thus corresponds to the endogenous decay rate. It was further assumed, that this reaction is active from the beginning of the experiment to the end. The OUR_{OHO} was modelled with Eq. 2.8 and an endogenous residue fraction f_U = 0.2 (Dold et al. 1980). Since no nitrification inhibitor was used, the oxygen uptake with respect to nitrification of nitrogen that was released during the degradation of biomass has to be considered. The fraction of nitrogen released was determined by using the nitrate concentration that was built up during the experiment in relation to the COD that was consumed within the experiment (integral of measured OUR). On average the nitrogen fraction (f_N) was 0.063 g N/ g COD with respect to the degraded activated sludge. The OUR_{NO3} is modelled according to Ramdani et al. (2010):

$$OUR_{NO3}(t) = 4.57 \cdot f_N \cdot (1 - f_U) \cdot b_{OHO} \cdot X_{OHO}(0) \cdot e^{-b_{OHO} \cdot t} \quad (mg\ O_2/(l*h)) \qquad \text{Eq. 2.13}$$

The measured OUR is given by:

$$OUR_{model} = OUR_{STOR} + OUR_{OHO} + OUR_{NO3} \quad (mg\ O_2/(l*h)) \qquad \text{Eq. 2.14}$$

To fit the measured OUR data with the model of Eq. 2.14 the parameter of q_{STOR}, X_{STOR}, b_{OHO} and X_{OHO} were obtained by nonlinear regression parameter estimation and are summarized for all batch tests in Table 2.3. Eq. 2.1 can now be rewritten:

$$X_{ORG} = X_{STOR} + X_{OHO} + X_{ORG,U} \quad (mg\ COD/l) \qquad \text{Eq. 2.15}$$

The fraction of stored substrate f_{STOR} and the fraction of active biomass F_a where obtained by

$$f_{STOR} = \frac{X_{STOR}(0)}{X_{ORG}(0)} \qquad \text{Eq. 2.16}$$

and

$$F_a = \frac{X_{OHO}(0)}{X_{ORG}(0)} \qquad \text{Eq. 2.17}$$

respectively.

As opposed to Henze et al. (2000), not a constant f_{cv} conversion factor was used in this investigation. The f_{cv} factor was calculated for the particular experiments as shown in Table 2.3 from the ratio of the integral of measured OUR to the VSS degraded. However, this f_{cv} conversion factor was not constant for all experiments but represents directly the COD of the degraded organic material.

The following conclusions can be drawn from the modelling results:

2.3.2.1 Degradation of X_{STOR}

The degradation rate of stored compounds q_{STOR} was observed to fall between 0.8 and 6.6 d^{-1} with a mean of 2.4 d^{-1}. This value matches well the magnitude of the hydrolysis rate parameter of activated sludge models. The fraction f_{STOR} was in average 2.1 % for the examined activated sludge. One sludge sample exhibited a higher portion of 6.9 %. No correlation of q_{STOR} to b_{OHO} was found. It is suggested that the magnitude of X_{STOR} is not a result of the F/M-ratio of the particular sludge, but rather that of the treatment process in the WRRF.

2.3.2.2 Initial heterotrophic $OUR_{OHO}(0)$

Modelling the OUR_{OHO} of the first days of the batch test as suggested makes it possible to identify the true initial endogenous heterotrophic respiration rate ($OUR_{OHO(0)}$) of the particular activated sludge, by excluding OUR_{STOR}.

Table 2.3: Results of the aerobic digestion batch tests

Nr.	Time			COD		N	Storage			Decay			
	Test	phase 1	phase 2	balance	f_{cv}	content	q_{STOR}	X_{STOR}	f_{STOR}	b_{OHO}	X_{OHO}	$OUR_{OHO}(0)$	F_a
	d	d	d	%	g O_2/g VSS	% COD	1/d	mg/l	%	1/d	mg/l	mg O_2/(l*h)	%
1	12	2.0	3.4	105	1.15	7.6	1.9	46	1.3	0.155	1446	7.5	54
2	15	1.5	4.0	104	1.37	5.8	2.4	30	0.9	0.132	1677	7.4	55
3	14	0.8	3.8	104	1.34	6.5	4.1	16	0.5	0.218	1523	11.0	54
4	21	1.8	3.2	105	1.29	7.2	1.9	33	1.1	0.125	1367	5.7	51
5	21	4.2	5.5	103	1.43	5.6	1.1	225	6.9	0.136	1701	7.7	52
6	18	2.3	5.0	102	1.52	6.9	1.9	94	3.2	0.101	1823	6.1	61
7	13	0.5	3.0	105	1.38	6.9	6.6	11	0.3	0.167	1873	10.4	59
8	32	1.5	6.2	102	1.52	5.4	3.2	16	0.8	0.121	1428	5.7	45
9	50	2.0	10.0	102	1.51	5.0	1.8	47	1.0	0.094	2499	8.0	56
10	30	2.1	5.0	101	1.57	5.9	1.7	65	1.4	0.125	2702	10.8	57
11	22	4.5	22.0	104	1.46	5.0	1.0	137	3.6	0.081	2285	5.7	58
12	30	5.0	30.0	104	1.50	6.3	0.8	172	3.8	0.077	2260	5.8	50

2.3.2.3 Heterotrophic decay rate b_{OHO}

According to Eq. 2.10, the heterotrophic decay rate b_{OHO} corresponds to the ratio of the heterotrophic endogenous respiration rate and the degradable fraction of the activated sludge. From the presented data this ratio is not constant between samples and therefore b_{OHO} is not constant. This observation is different from the theory of Marais et al. (1976), van Haandel et al. (1998) and Ramdani et al. (2010). There are two ways to explain this observation:

(A) Regarding X_{OHO} from the substrate point of view

With an decreasing ratio of OUR to active biomass (X_{OHO}), which occurs in low F/M-ratio systems, degradability characteristics of heterotrophic cell mass in activated sludge reduces, so that the rate of degradation (endogenous decay rate) decreases.

(B) Regarding X_{OHO} from the organisms point of view

Activated sludge is a highly complex ecosystem and accommodates living organisms that have many strategies to deal with changing environmental conditions. Therefore it is rather likely that organisms from low F/M systems are more prepared to deal with starvation and thus die slower with a reduced endogenous decay rate.

2.3.2.4 Correlation of b_{OHO} to $spOUR_{OHO}(0)$

From the empirical point of view the measured endogenous decay rate parameters are shown in Figure 2.4 in relation to the specific endogenous respiration rate of X_{OHO} at the beginning of the experiment with respect to the concentration X_{ORG}. Note, that the results from experiment 08A was considered an outlier.

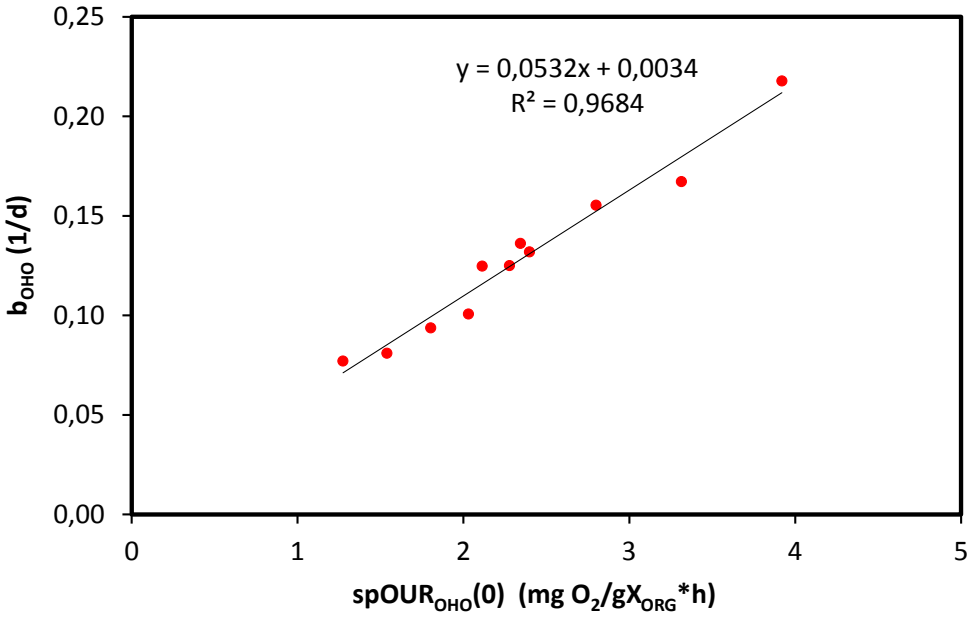

Figure 2.4: Correlation of b_{OHO} and $spOUR_{OHO}(O)$

From the slope (0.053) of the line that fits the data best an estimate of the degradable fraction ($(1-f_U)*X_{OHO}$) of X_{ORG} can be derived by inserting Eq. 2.2 into Eq. 2.10 and converting hours to days as well as grams to milligrams.

$$\frac{OUR_{OHO}(0)}{X_{ORG}(0)} \cdot \frac{24}{f_{DEG} \cdot 1000} = b_{OHO} \qquad (d^{-1}) \qquad \text{Eq. 2.18}$$

Then in this batch of experiments the degradable fraction of X_{ORG} is:

$$f_{DEG} = \frac{24 \cdot 100}{0{,}053 \cdot 1000} = 45{,}3\% \qquad \text{Eq. 2.19}$$

From the data of this investigation it can be concluded that X_{OHO} is rather constant and the decay rate varies with loading conditions. This is in contrast to the current theory that the decay rate b_{OHO} is constant and the active biomass in terms of X_{OHO} varies with loading conditions of the activated sludge.

Adding the portion of degraded stored material of in average 2.1% a total degradable fraction of about 47% of X_{ORG} can be calculated from the presented data. This degradable fraction lies within the range as reported for aerobic degradation of activated sludge in Metcalf and Eddy (2004). It is assumed, that degradation rates

exceeding 47% are due to further adaptation of X_{OHO} to starvation and the activity of predators. This additional degradation takes place in the phase 3 of the aerobic digestion batch experiment.

2.4 Conclusion

The objective of this study was to find out whether the analysis of endogenous respiration profiles lead to a constant decay rate on the basis of the degradation of a homogenous degradable activated sludge fraction. From the observed data and the modelled results the following can be concluded:

1. A structural analysis of endogenous OUR profiles revealed that the endogenous decay during the first two days is overlain by a faster reaction that degrades most likely stored substrate.

2. In most of the experiments after 5 days of digestion the exponential decrease of OUR slows down, does not show an exponential behaviour and can even increase temporarily. This behaviour is thought to be the consequence of proliferation of higher organisms and adaptation of active biomass to the conditions of severe starvation.

3. The degradable material of the activated sludge can be clearly separated into two fractions; that is easily degradable storage compounds X_{STOR} and slower degradable active heterotrophic biomass X_{OHO}.

4. In general the values of b_{OHO} in this study were smaller than reported elsewhere in the literature.

5. The decay rate b_{OHO} of X_{OHO} was not as constant between samples as regarded in the current theory of activated sludge modelling. In this study it was found that b_{OHO} decreases in a strong correlation with a decreasing activity of the activated sludge expressed as the ratio of the $OUR_{OHO}(0)$ to $X_{ORG}(0)$. An important consequence of this correlation is that the fraction of X_{OHO} in the examined activated sludge remains rather constant over a wide range of loading conditions.

2.5 Pitfalls in determining the decay rate parameter

2.5.1 Suppression of nitrification with ATU

The determination of the decay rate of OHOs by respirometry involves the consideration of ammonia oxidation, because ammonia is released in the course of degradation of organic cell material. This can be achieved by either suppressing nitrification using a nitrification inhibitor, commonly allylthiourea (ATU) or by modelling the oxygen consumption due to nitrification during the degradation batch experiment.

In a first attempt to avoid the modelling approach, ATU was tried as nitrification inhibitor. Reviewing the literature for a suitable ATU concentration that should be used produced no reliable information. The only substantial source is Henze et al. (1987), where a dosage of ATU is recommended to achieve 20 mg ATU/l. However, an aerobic degradation batch experiment lasts for at least three to four weeks. So it was the question whether ATU would suppress nitrification during the entire experimental time.

An experiment was set up with four reactors containing activated sludge from a municipal wastewater treatment plant with a volume of 2.5 l. The TSS concentration was diluted to 3.0 g TSS/l. The first reactor was operated without ATU dosage, where the other reactors where spiked with ATU at the beginning of the batch experiment with 5, 10 and 60 mg ATU/l, respectively. All the reactors were aerated between a lower (4.0 mg O_2/l) and an upper limit (6.0 mg O_2/l) and the oxygen uptake rate was measured. OUR was monitored over a time of 6 days.

The results are presented in Figure 2.5 and show that in the reactor spiked with 5 mg ATU/l nitrification suppression stopped even before one day, whereas the reactor spiked with 10 mg ATU/l stopped to suppress nitrification at 2.5 days. However, the reactor containing 60 mg ATU/l did not show a loss of nitrification suppression during the experimental time. The termination of nitrification suppression is indicated by an increase of OUR due to the accumulated ammonia that could not be oxidised during effective nitrification suppression. From these results it is concluded that ATU is used up metabolically and gives only a time restricted protection against nitrification.

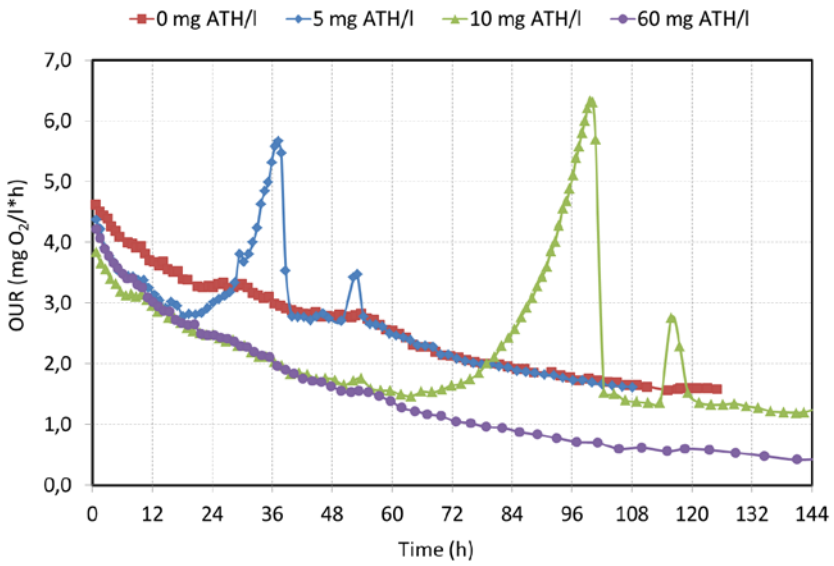

Figure 2.5: ATU degradation in aerobic degradation batch experiments

A further result of this test was the observation of differences of the decrease of OUR in the four reactors. This is very important because the characteristic OUR decrease is directly used to determine the decay rate parameter. To investigate the possible reduction of aerobic respiration of OHO as a function of the ATU concentration in the aerobic degradation batch experiment the above described experiment was repeated with different ATU concentrations at the beginning of the batch test according to Figure 2.6.

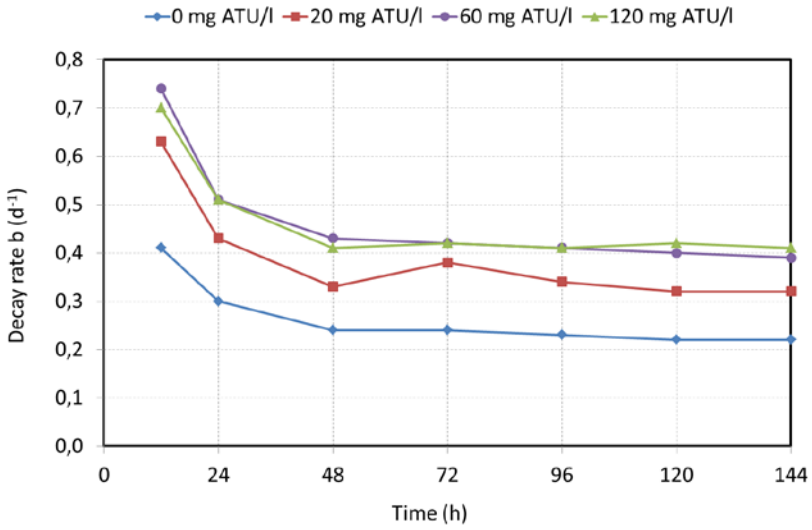

Figure 2.6: Decay rate of OHO evaluated for every day in an aerobic degradation batch experiment

The decay rate parameter was calculated by linearization of the OUR data for every single day of the experiment. The results show for 20 mg ATU/l a significant acceleration of decay. With 60 mg ATU/l the decay rate after 6 days of test time the decay rate was close to double of the decay rate without ATU spiking. A further increase of the decay rate with 120 mg ATU/l could not be observed.

Furthermore in all tests, with or without ATU dosage the decay rate decreased strongly in the first two days and remained constant for the rest of the experimental time.

Taking the aerobic utilisation of ATU into account as well as the suppression not only of the nitrifying organism but also the reduction of the aerobic metabolism of OHO, the use of ATU within aerobic degradation experiments to obtain the decay rate parameter cannot be recommended and was not used throughout this project.

2.5.2 Using open water surface respirometer

Respirometry in an aquatic system uses the measurement of the oxygen uptake rate. Thereby it is intended to observe only the reduction of dissolved oxygen exclusively due to respiration of microorganisms. The range of oxygen concentration is set sufficiently high (e.g. 4 - 6 mg O_2/l) to guarantee that oxygen is not limiting the biochemical reaction.

However, using an open water surface respirometer the observed oxygen uptake rate is decelerated by a constant oxygen intrusion from the atmosphere via the open water surface to the mixed liquor (see Figure 2.7). The oxygen intrusion for a reactor of a diameter of 0.13 m and a mixed liquor volume of 2.5 l in this project was measured at 1.0 mg O_2/(l*h).

The example of OUR profiles of a simulated aerobic degradation experiment for a closed and an open respirometer is shown in Figure 2.7. This example is based on an ideal exponential (R^2 = 1.00) decrease of OUR in an open respirometer and a decay rate of $b = 0.24\ d^{-1}$ (see Figure 2.8). However, considering the constant oxygen intrusion over the experimental time in the open respirometer the closed respirometer would have not the ideal exponential degradation characteristic, in particular at the

end of the experiment, where the OUR of the closed respirometer is multiple of that of the open respirometer.

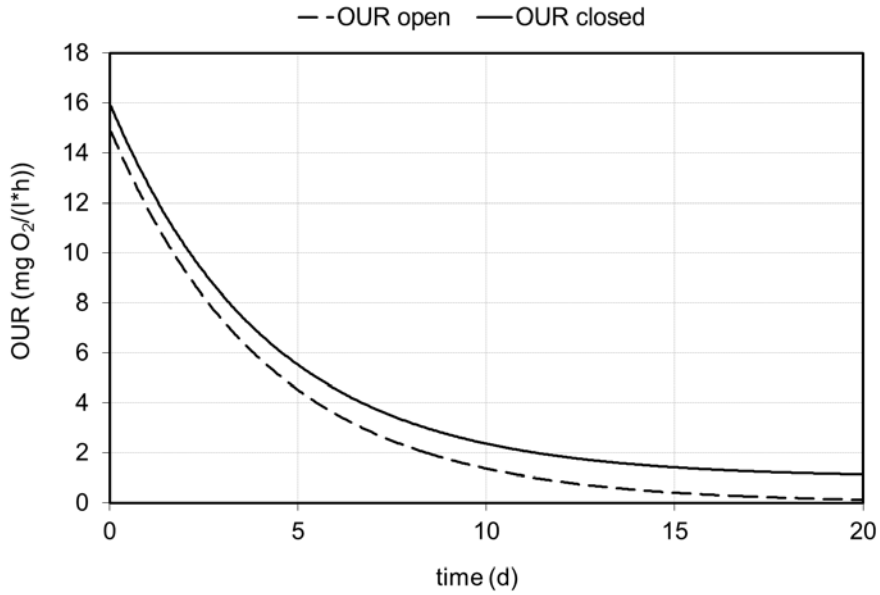

Figure 2.7: Example of OUR measurements with an open water surface respirometer and a closed respirometer configuration

The linearization would yield for the closed respirometer an R^2 of 0.95 for a first order reaction kinetics (Figure 2.8), which is generally accepted as sufficiently high for proving the validity of a first order reaction. However, the first order reaction rate has a much lower value (b = 0.134 d^{-1}).

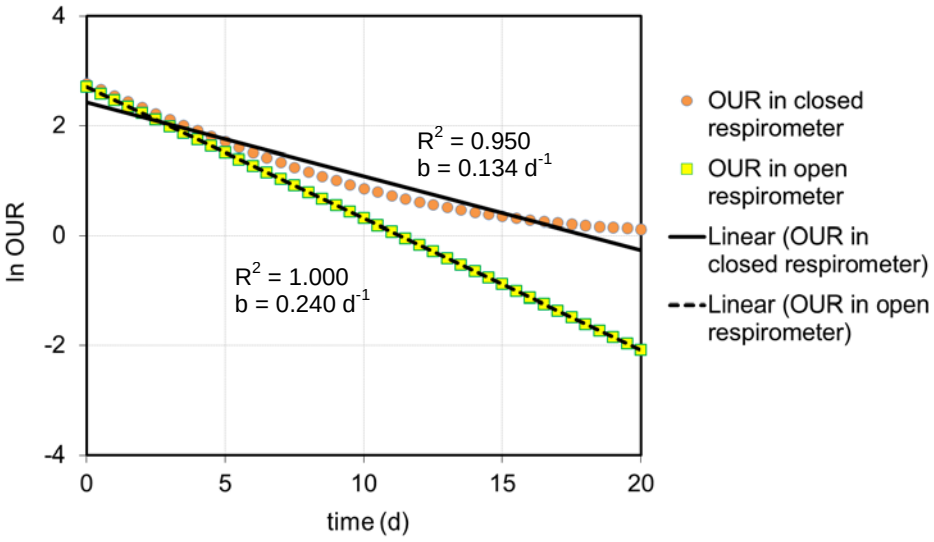

Figure 2.8: Linearized OUR measurements

The conclusion of this observation is that the decay rate determined in an open respirometer generally is much higher compared to the measurement in a closed respirometer. Oxygen intrusion obviously generates a systematic error which can bend the non-exponential OUR decrease to a more ideal exponential characteristic. This is surprising, but could explain partly why the decay rate determinations based on OUR measurements in open respirometers produced rather high values.

3. Physiological adaptation of growth kinetics in activated sludge

3.1 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. 3.1) 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):

$$\mu = \mu_{max} \cdot \frac{S}{K_S + S} \qquad (d^{-1}) \qquad \text{Eq. 3.1}$$

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.

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 (van Loosdrecht et al., 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 et al., 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 to 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. 3.2 (Herbert, 1958; McKinney, 1960). It considers oxygen consumption due to substrate utilisation resulting in microbial growth and oxygen consumption due to endogenous respiration.

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

Eq. 3.2

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); Henze et al. (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 3.1) 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.

Table 3.1: $\mu_{max,OHO}$ values for low and high S/X ratio methods

	low S/X	high S/X
	d^{-1}	d^{-1}
Kappeler and Guyer (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 et al. (1993)	1.5	
Pollard et al. (1998)	0.8	4.0

Third, reviewing the literature there is an extensive knowledge addressing physiological adaptation of bacterial cultures. In a historical perspective Jannasch et al. (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), Daigger and

Grady (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 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?

3.2 Materials and Methods

3.2.1 Notation

This investigation deals exclusively with the kinetic growth properties of ordinary heterotrophic organisms (OHOs). 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.

3.2.2 Characteristics 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 water resource recovery facility (WRRF) situated in the Northeast of Germany ranging from 15000 to 600000 population equivalents (PE). All plants were fully nitrifying and performed extensive denitrification.

Table 3.2: 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	200000	2830	68	13.8	4.9	20
B	600000	3020	74	20.3	6.7	11
C	80000	3040	77	14.5	4.8	70
D	15000	2663	80	25.8	9.7	13
E	50000	2810	69	20.4	7.3	30
F	400000	3060	77	17.5	5.7	17

With the exception of plant C and D all WRRFs 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 3.2. 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.

3.2.3 Experimental setup

The experimental setup and the experimental approach are shown in Figure 3.1. 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).

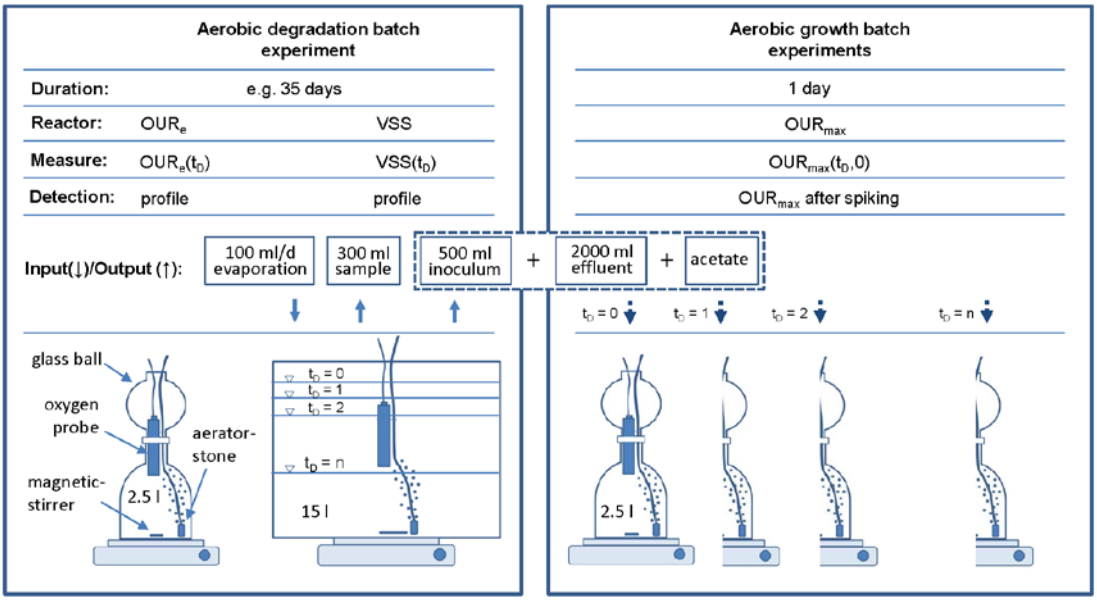

Figure 3.1: Experimental Setup

The identically 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 to 2 days conducting the growth tests. The operation mode was such that in the first week every day and then in increasing time intervals 500 ml inoculum from the VSS-reactor was withdrawn, diluted with effluent water from a WRRF 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).

3.3 Parameter Determination

3.3.1 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 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 to 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 Figure 3.2.

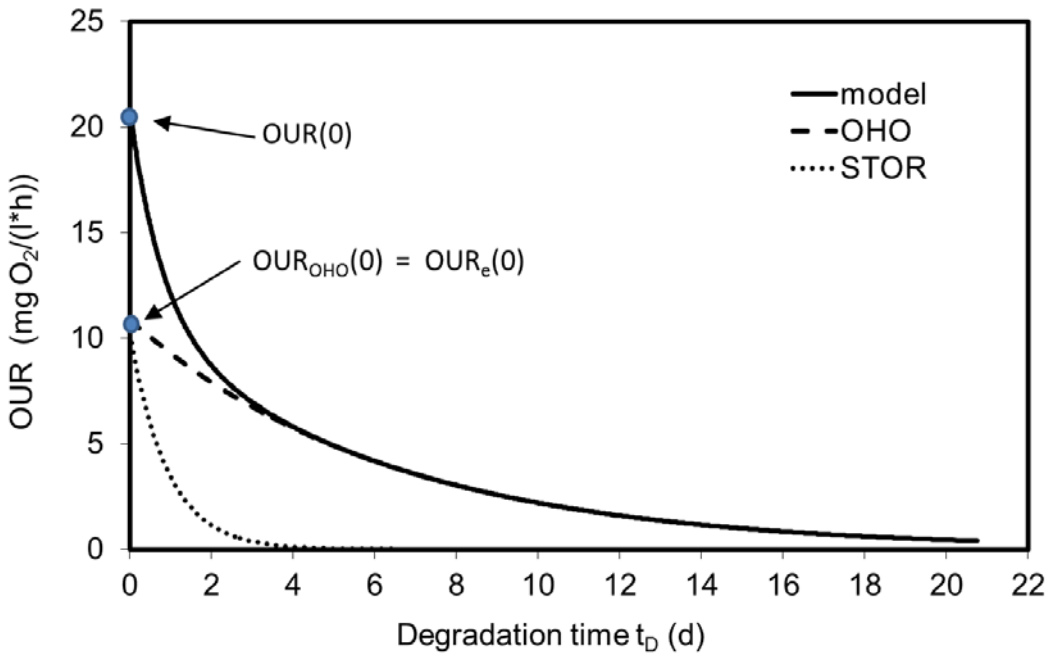

Figure 3.2: Respirogram with the degradation of X_{STOR} and X_{OHO}

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 to 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.

3.3.2 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.

3.3.3 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 Figure 3.3).

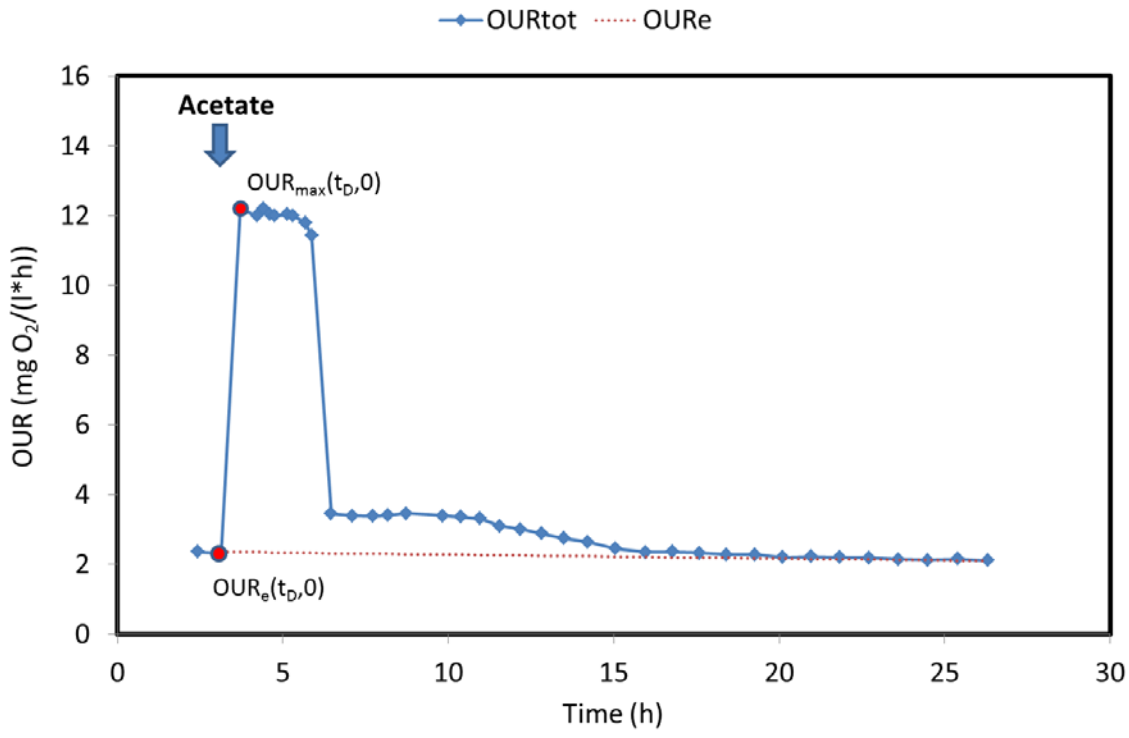

Figure 3.3: Respirogram of a growth experiment

The added amount of acetate was selected to increase the substrate saturation term from the Monod kinetics to unity. At this point μ equals μ_{max}. The $OUR_{max}(t_D,t_G)$ in the growth test over the time of substrate saturation is expressed with Eq. 3.3:

$$OUR_{max}(t_D,t_G) = \left[\frac{(1-Y)}{Y}\cdot\mu_{max}\cdot X_{OHO}(t_D,0)\cdot e^{\mu_{max}\cdot t_G} + (1-f_U)\cdot X_{OHO}(t_D,0)\cdot b_e\cdot e^{-b_e\cdot t_G}\right]/24$$

(mg O_2/(l*h)) Eq. 3.3

This equation is based on Eq. 3.2 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 et al., 1976; Ramdani et al., 2010). For the assessment of μ_{max} Eq.3.3 is only of interest for t_G = 0, which is the time of spiking the activated sludge with acetate. At the time of the first OUR measurement μ_{max} is already identifiable. For t_G = 0 Eq. 3.3 reduces to:

$$OUR_{max}(t_D,0) = \left[\frac{(1-Y)}{Y}\cdot\mu_{max}\cdot X_{OHO}(t_D,0) + (1-f_U)\cdot X_{OHO}(t_D,0)\cdot b_e\right]/24$$

(mg O_2/(l*h)) Eq. 3.4

Rearranging Eq. 3.4 yields:

$$\frac{OUR_{max}(t_D,0)\cdot 24}{X_{OHO}(t_D,0)} = \frac{(1-Y)}{Y}\cdot\mu_{max} + (1-f_U)\cdot b_e \qquad (d^{-1}) \qquad \text{Eq. 3.5}$$

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. 3.6

$$OUR_e(t_D,0) = (1-f_U)\cdot X_{OHO}(t_D,0)\cdot b_e/24 \qquad (mg\ O_2/(l*h)) \qquad \text{Eq. 3.6}$$

Rearranging Eq. 3.6 for $X_{OHO}(t_D,0)$ yields

$$X_{OHO}(t_D,0) = \frac{OUR_e(t_D,0)\cdot 24}{(1-f_U)\cdot b_e} \qquad (mg\ X_{OHO}/l) \qquad \text{Eq. 3.7}$$

Introducing Eq. 3.7 into Eq. 3.5 leads to:

$$\frac{OUR_{max}(t_D,0)\cdot(1-f_U)\cdot b_e}{OUR_e(t_D,0)} = \frac{(1-Y)}{Y}\cdot\mu_{max}+(1-f_U)\cdot b_e \qquad (d^{-1}) \qquad \text{Eq. 3.8}$$

Resolving Eq. 3.8 to μ_{max} gives an expression according to Eq. 3.9 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}.

$$\mu_{max} = \frac{Y}{(1-Y)}\cdot(1-f_U)\cdot b_e\cdot\left(\frac{OUR_{max}(t_D,0)}{OUR_e(t_D,0)}-1\right) \qquad (d^{-1}) \qquad \text{Eq. 3.9}$$

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)

3.4 Presentation and Repetition of Experiments

To illustrate the dependency of growth and decay the results of degradation test B and C are displayed graphically (Figure 3.4 and Figure 3.5), whereas in summarizing considerations the results of all tests are discussed and presented in Table 3.3 and Figure 3.6 and Figure 3.7. 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.

3.5 Results and Discussion

3.5.1 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 (Figure 3.4 (a) and (b)) the rate parameter b_e and b_{max} were derived as described. According to Table 3.3 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.

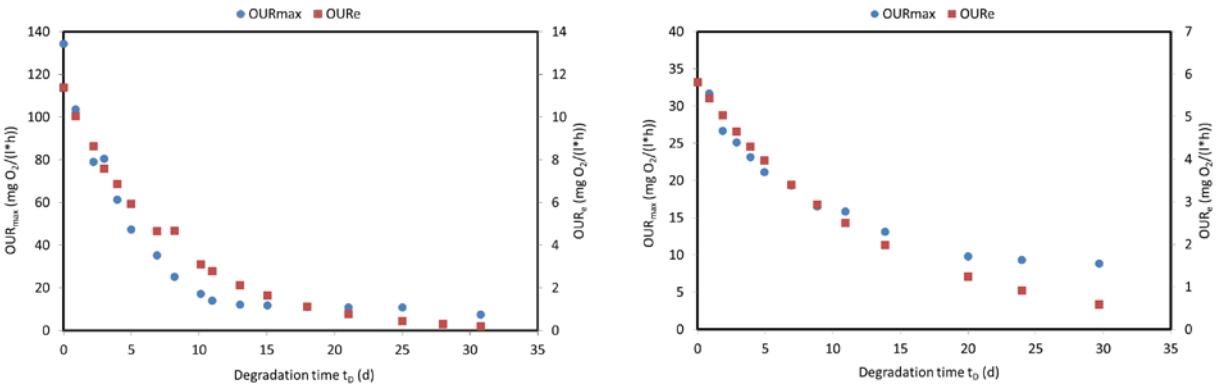

Figure 3.4: (a) OUR_e and OUR_{max} of test B.1 and (b) OUR_e and OUR_{max} of test C.1

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 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 3.3 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).

Table 3.3: Respiratory and kinetic results of degradation experiments (R^2 in brackets)

Test	Test-	OUR_{max}	μ_{max}	OUR_e	b_e	b_{max}			
	Time	$t_D = 0$				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

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 WRRF 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 Figure 3.4 (a) from day 5 to 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}.

3.5.2 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 Figure 3.4) and relating both values to each other generates the basis for the determination of μ_{max} according to Eq. 3.9. This implies furthermore that a variation of μ_{max} as documented in Figure 3.5 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 Figure 3.5 (a) 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 3.3. 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.

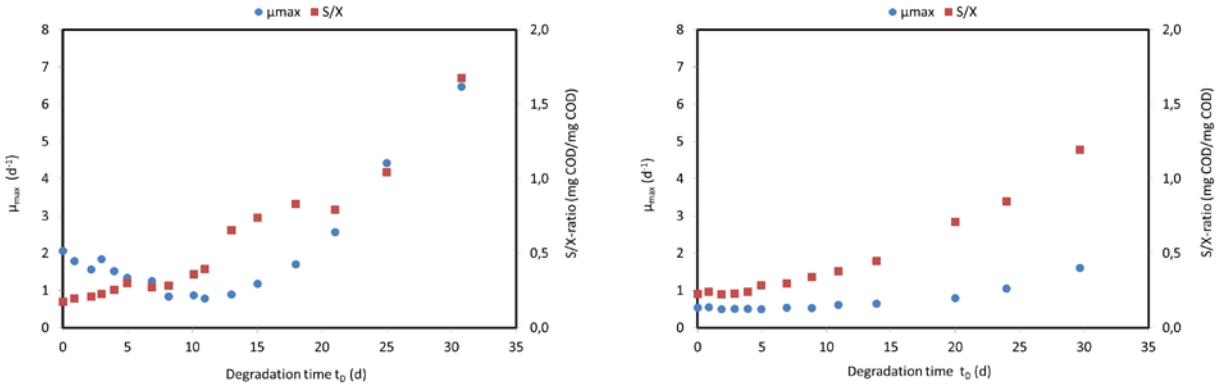

Figure 3.5: (a) μ_{max} and S/X-ratio of test B.1 and (b) μ_{max} and S/X-ratio of test C.1

In contrast to this, the high SRT sludge in Figure 3.5 (b) 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 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 fate 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}.

3.5.3 Maximum specific growth rate as an intrinsic property

From the experimental data of Figure 3.5 (a) 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 et al., 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).

3.5.4 Relation of μ_{max} and b_e

Looking at the initial values (t_D = 0) of μ_{max} and b_e (Figure 3.6) which are characteristic for the activated sludge as it was taken from WRRF 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.

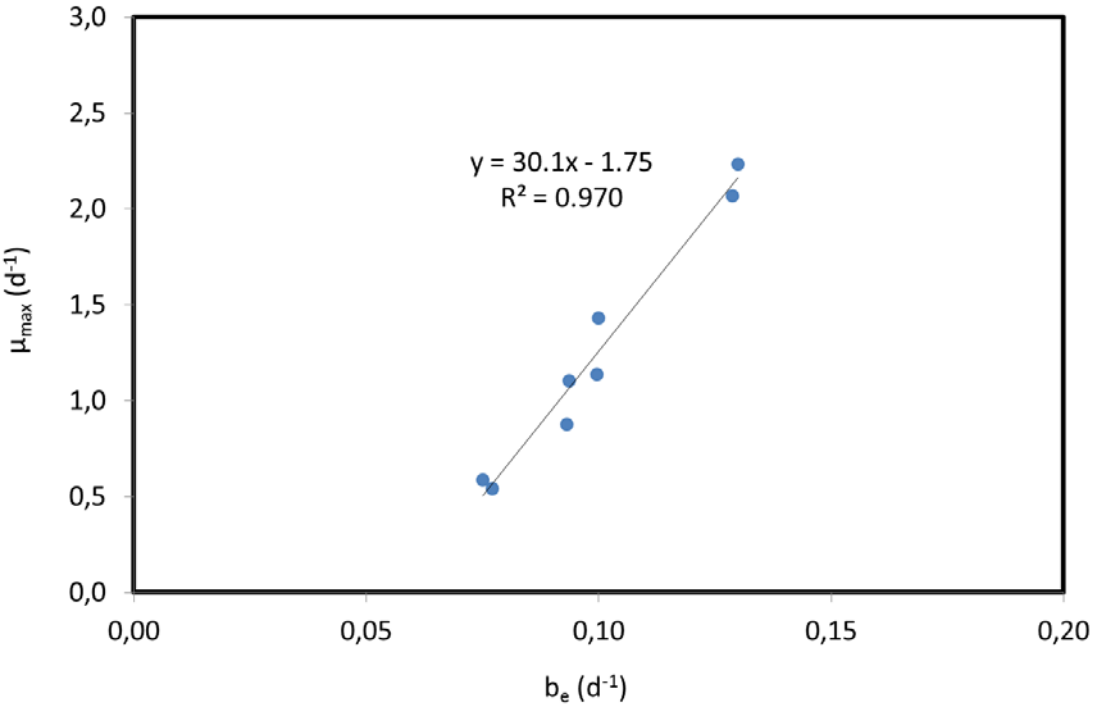

Figure 3.6: Relation of μ_{max} and b_e

An explanation can be formulated as follows: In an engineered environment such as a high loaded system activated sludge bacteria 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.

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 et al. (1976) for pure cultures of Klebsiella aerogenes.

These experimental results are notable because in current 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. (2012), Friedrich et al. (2016) 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 a 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%.

3.5.5 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:

$$PSF = \frac{\mu_{max}}{b_e} \quad (-) \qquad \text{Eq. 3.10}$$

A high PSF describes a microbial community that is physiologically growth-optimized, whereas a low PSF describes a microbial community that is physiologically survival-optimized.

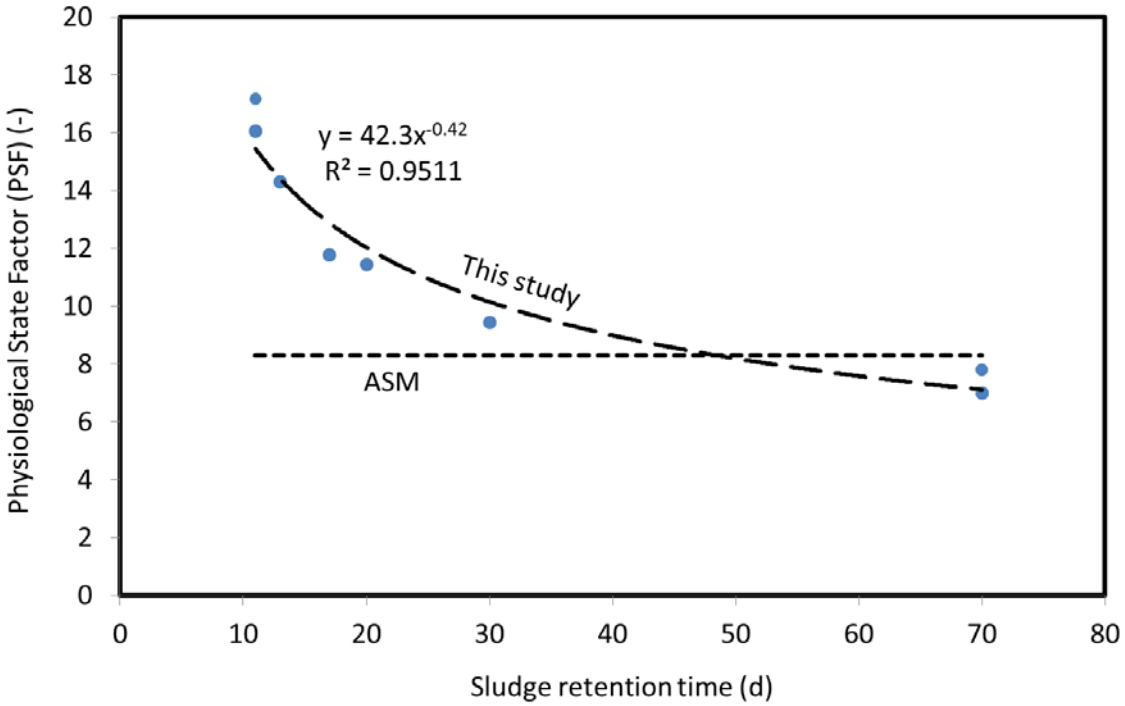

Figure 3.7: Correlation of the PSF and SRT

Figure 3.7 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. 3.2 as representative for the existent ASMs would yield into a horizontal line with a constant value of PSF = 2.0/0.24 = 8.3.

3.6 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 characteristics 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 WRRF.

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.

3.7 Experimental identifiability of μ_{max} of OHOs

3.7.1 Existing procedure to determine μ_{max}

The unlimited, exponential growth of bacteria can be observed in an environment, containing all essential compounds for growth. Thereby, the particular substrate which serves as energy source is most predominant for the physiology of the bacterial cell.

The specific maximum growth rate (μ_{max}) of bacteria as described by Monod (1949) (see chapter 3.1) corresponds to the first order reaction parameter in the presents of excess substrate concentration, since the saturation term of the Monod Equation approaches unity.

$$\frac{dX_{OHO}}{dt} = \mu_{max} \cdot \frac{S}{K_S + S} \cdot X_{OHO} \qquad \text{Eq. 3.11}$$

If $S >> K_S$ then $\frac{S}{K_S + S} \approx 1$

Equation 3.11 can be simplified to:

$$\frac{dX_{OHO}}{dt} = \mu_{max} \cdot X_{OHO} \qquad \text{Eq. 3.12}$$

$$X_{OHO}(t) = X_{OHO}(0) \cdot e^{\mu_{max} \cdot t} \qquad \text{Eq. 3.13}$$

For the determination of μ_{max} in activated sludge problems arise by the fact that $X_{OHO}(0)$ cannot be measured directly and therefore has to be modelled (see e.g. WRC, 1984 or Lee et al, 2006). Using Eq. 3.9 from chapter 3.3.3 generates a value for μ_{max} which is based on the decay rate as well as the ratio of endogenous OUR before and the maximum OUR after spiking of substrate in excess. However, using this procedure for parameter identification no exponential OUR increase can be observed. But this would be essential to identify experimentally a true first order reaction. Without an exponential increase of OUR, the observed growth does not has to be necessarily an increase of bacterial cells in number. It could stand as well for an increase of cell volume and cell size.

Taking this into account it is questionable whether the identifiability of the specific maximum growth rate of OHOs with the described method is sufficiently high to use this parameter in ASM.

3.7.2 Investigating the exponential growth potential in activated sludge

Degradation experiment

This investigation is mainly based on the degradation experiment B2 that is executed as described in chapter 3.2. For comparing low and high SRT growth behaviour results from the degradation test C2 are introduced in chapter 3.7.3. The history of B2 is best described by the endogenous respiration profile according to Figure 3.8. For modelling of OUR components see chapter 2.3.2.

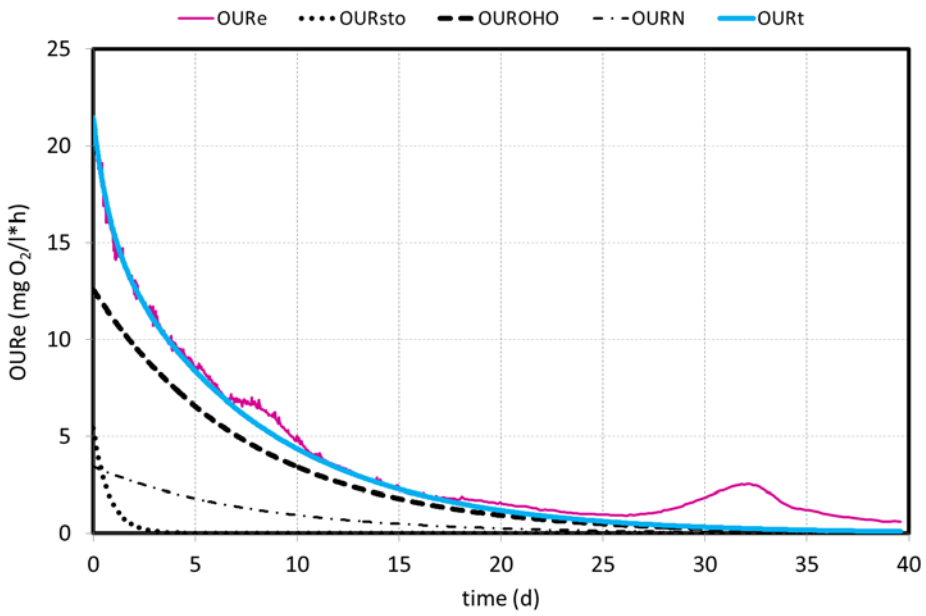

Figure 3.8: Endogenous respiration profile of degradation experiment B2, where OUR is the measured OUR and OURt is the sum of the modelled OUR due to respiration of stored material (OURsto), endogenous respiration of OHO (OUR_{OHO}) and oxygen consumption due to nitrification (OUR_N)

Within this experiment after two days the degradation of X_{STOR} was terminated. From day 7 to 11 the endogenous respiration was characterised by a higher OUR_e, which could not be explained by the respiration of OHOs and oxygen consumption due to

nitrification. Similar to other experiments at this stage of the aerobic digestion a mass development of higher organism could explain this irregularity. From day 17 to 27 the measured OUR was slightly higher than predicted and from then on OUR increased until day 32 up to three times the OUR value of day 27. However, at this late time of the degradation experiment higher organisms never occurred in the mixed liquor. Therefore microbial adaption to a particular substrate pool would rather explain this observation. After this second irregularity OUR_e decreased until the end of the experiment.

Growth experiments

From the mixed liquor of the above described degradation experiments a sample was taken in regular intervals, diluted and the endogenous respiration observed in a closed respirometer configuration according to chapter 3.2. The sample then was spiked with acetate in a concentration of 532 mg COD/l and the OUR profile observed for initially 24 h and later 36 h (Figure 3.9).

At the beginning of the degradation experiment the endogenous respiration (1) is comparatively high. The activated sludge which is still rich in active biomass shows a high instantaneous OUR increase (2) after acetate spiking. However, from this high level OUR decreased slightly but showed a low increase (3) until acetate is depleted where OUR drops down to a lower endogenous level (4). After two days of aerobic digestion the instantaneous OUR increase after substrate spiking was already significantly lower but the characteristic of the plateau until substrate depletion was the same. Notably the increase on the high OUR level (3) followed a first order reaction rate with an R^2 of 0.97 and was already higher on the second day than for the undigested activated sludge.

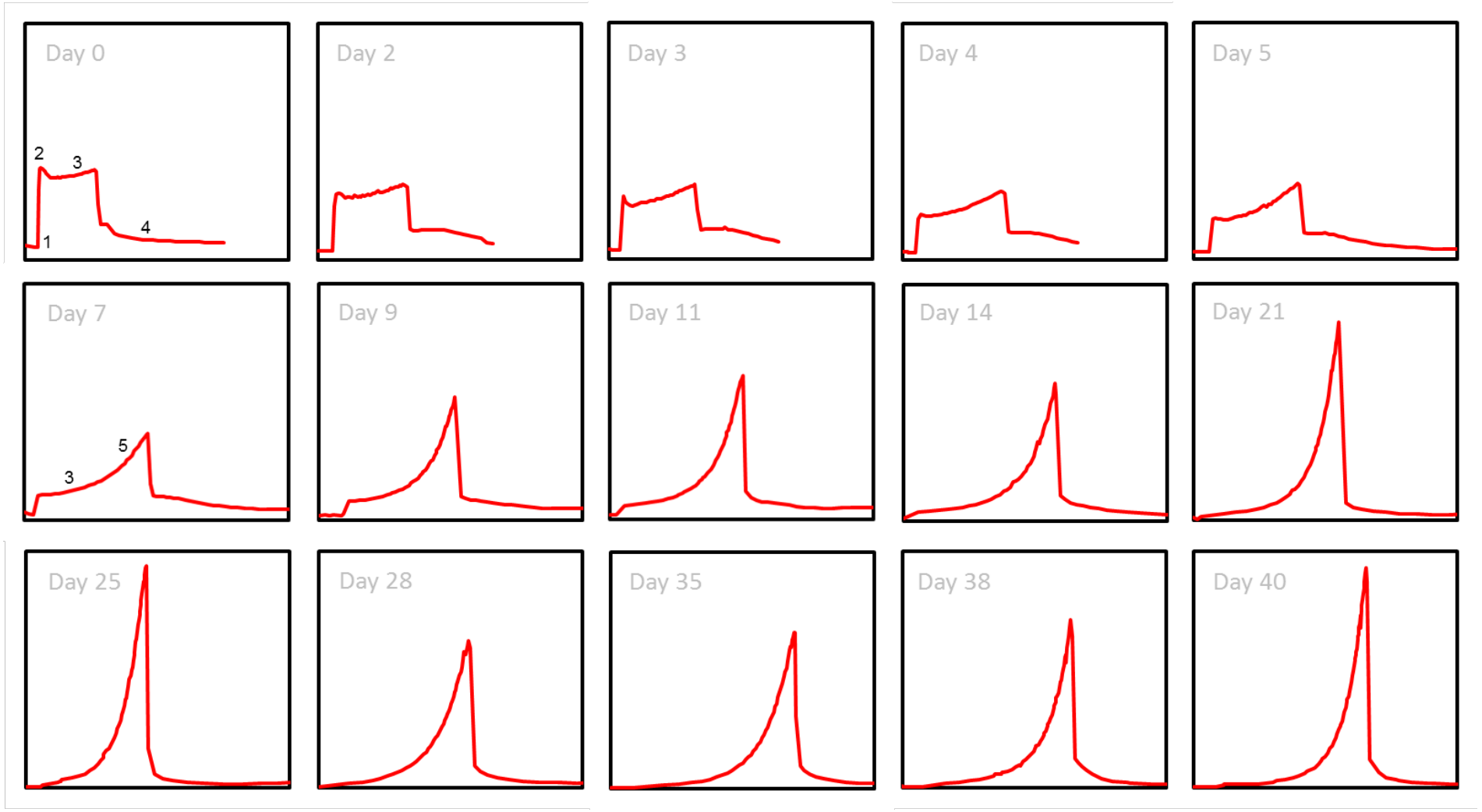

Figure 3.9: OUR profile of growth experiments where the maximum of x-axis is t = 36 h and the maximum of y-axis is OUR = 80 mg O_2/(l*h)

With progressive aerobic digestion time the initial high OUR level (2) due to substrate spiking became lower, the initial decrease disappeared and the following increase (3) became more dominant. However, the linearization after day 7 indicates that there are two different reaction rates for 3 and 5 (Figure 3.9) that could be found by a separate linearization (Figure 3.10).

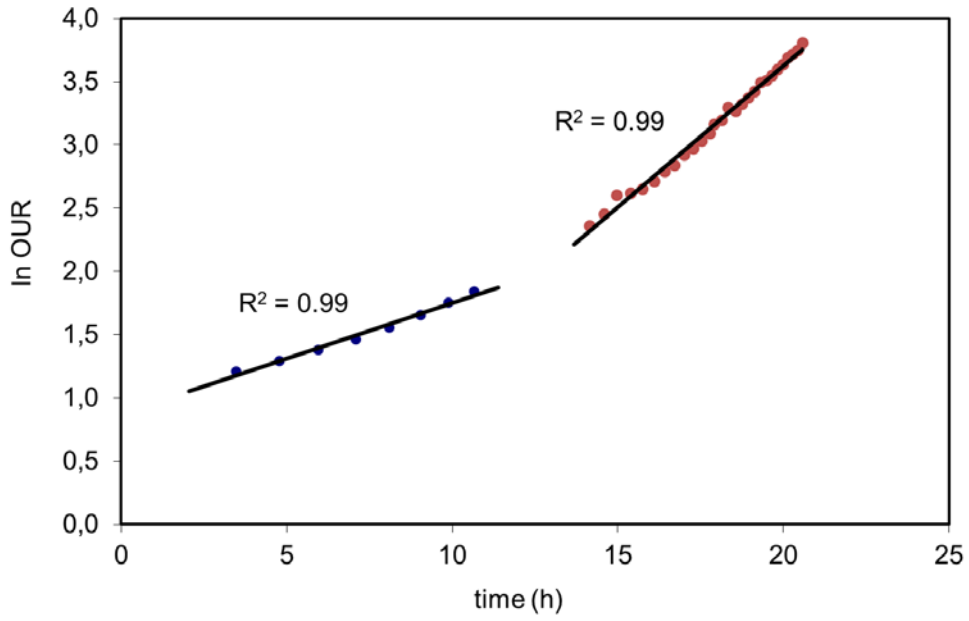

Figure 3.10: Linearization of the exponential OUR increase after substrate spiking, here for a sludge sample from day 4 of the degradation experiment

The first slow reaction is identifiable from hour 3 to 10 of the growth experiment. The second faster reaction is identifiable from hour 14 to the depletion of substrate. The R^2 of both reactions was throughout > 0.99. In that way it was found that there are two exponential growth processes, which by trend increased with the time of digestion. It is hypothesised that the first reaction with the exponential reaction rate $\mu_{1,exp}$ corresponds to the rate of a lag phase (lag rate) of bacterial growth. The lag phase of microbial growth is the time where the macromolecular composition of the cell is brought to a level that the exponential growth of bacteria namely the second reaction ($\mu_{2,exp}$) is only limited by the limiting substrate concentration and not by the lack of cell constituents (Monod (1949); Rolfe et al. (2011)).

However, it is curious that both the lag rate and the exponential growth rate increase with advancing starvation (Figure 3.11). From experimental results an explanation is hypothesised as follows:

First of all recognising the endogenous respiration profile of Figure 3.8, there was a late increase and subsequent decrease of the endogenous OUR_e between day 30 and 35 which is possibly associated with a slight increase of X_{OHO}. As a consequence of the increase of X_{OHO} in the degradation experiment the S_0/X ratio decreases in the growth experiment. Now, looking at Figure 3.11 it is striking that both exponential growth rates decrease together with the S_0/X ratio at the respective range of time. From this perspective it would be possible that the S_0/X ratio influences the exponential growth rate (Chudoba et al., 1992).

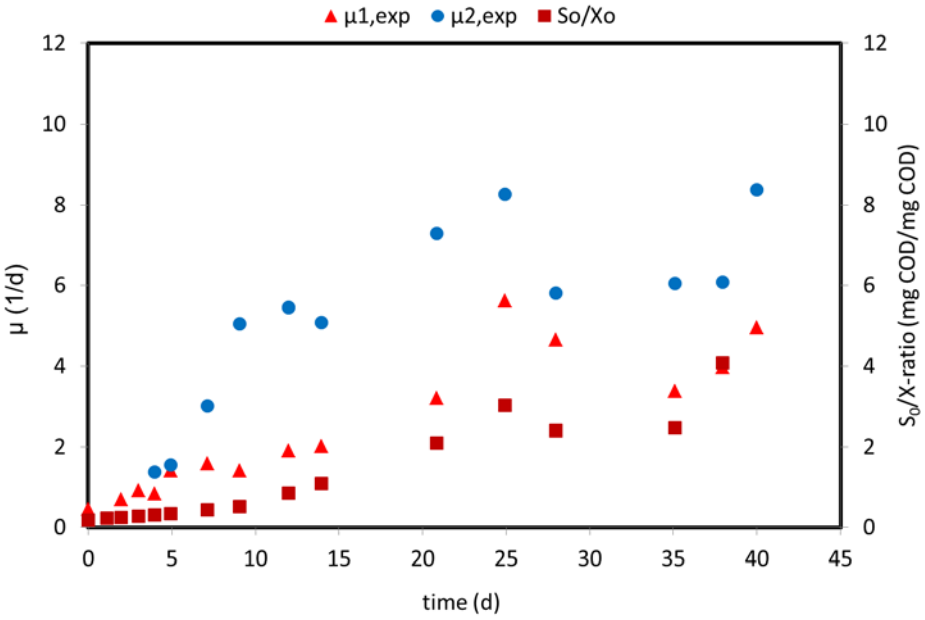

Figure 3.11: Exponential growth rates obtained from growth experiments with aerobically digested sludge samples from a degradation experiment together with the ratio of substrate to biomass at the time of substrate spiking.

Furthermore from these results it is obvious that an exponential growth cannot be observed in activated sludge as grown in a WRRF and therefore a specific maximum growth rate describing exponential growth is not identifiable using respirometry. From the point of physiology two aspects characterise a bacterial cell grown in an activated sludge process:

First, due to a general lack of substrate the cell itself has not fully developed the macromolecular machinery and needs to grow in size in order to multiply instantaneously in the presence of excess substrate.

Second, due to the alternating availability and absence of substrate within the activated sludge process the cell has developed the potential to store high amounts of substrate (Majone et al., 2007).

After substrate spiking OUR_{max} is an expression for the maximum potential to gain energy from an available substrate by respiration but not necessarily an expression for the maximum growth rate neither by growth in cell size nor due to cell multiplication. However, without the feast and famine pattern of substrate supply in a batch test and probably more important with progressive starvation bacteria lose the ability of storage. Substrate is directly and exclusively used for building up or complete cell constituents. Immediately after substrate spiking, OUR starts to increase with the lag rate and passes over to the exponential growth process by cell multiplication. Possibly both the increasing S/X_0 ratio and the loss of storage potential might be the reason for the increasing lag and growth rate.

3.7.3 Which growth rate should be used in ASM?

Even if the meaning of μ_{max} as described by Monod (1949) is based on exponential growth, it is the question whether it is compulsory to use μ_{max} only when it is derived from an exponential increase of a growth indicating parameter.

Figure 3.12 shows for test B2 (SRT = 11 days) and test C2 (SRT = 70 days) a comparison of $\mu_{max}(0)$ as it is derived from the initial $OUR_{max}(0)$ and $\mu_{max,exp}$ which is measured as an exponential increase of OUR after substrate spiking. The lag rate is omitted in the further discussion due to its proportionality to $\mu_{max,exp}$.

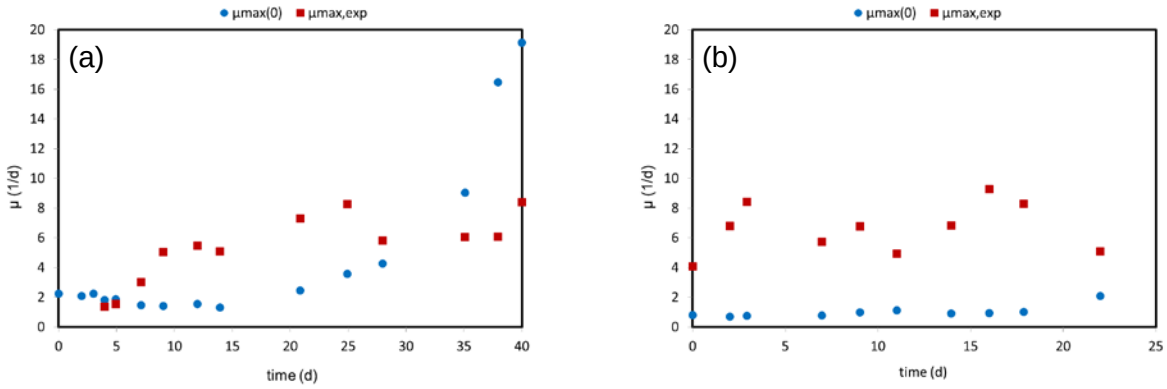

Figure 3.12: (a) $\mu_{max}(0)$ and $\mu_{max,exp}$ of test B.2 and (b) $\mu_{max}(0)$ and $\mu_{max,exp}$ of test C.2

The identifiability of $\mu_{max,exp}$ for test B2 starts at day 4 of the degradation experiment where $\mu_{max,exp}$ in test C2 is identifiable already in the undigested activated sludge. This is consistent with the suggestion that severe starvation as it occurs in activated sludge systems with very high SRTs might reduce the ability of substrate storage to a high degree.

More important the experimentally observed value of $\mu_{max,exp}$ is much higher than the partly derived value of $\mu_{max}(0)$. Which value describes the reality of OHOs more accurately? Recognising the nature of the bioassay and how μ_{max} is generated indicates that $\mu_{max}(0)$ is based on an instantaneous reaction of OHOs where on the other hand $\mu_{max,exp}$ is the result of the change of bacterial composition which lasts for several hours.

Furthermore, μ_{max} in general has little impact on predicting the fraction of OHOs in an activated sludge system with medium and high SRTs, since the substrate concentration is so low and the time for bacterial growth so long, that the growth yield dominates the magnitude of OHOs. However, this might be different for very low SRT (e.g. 0.5 days) systems. Using a method that generates a parameter in a period of time that is longer than the SRT is probably not reflecting reality.

From this perspective it is $\mu_{max}(0)$ that is in a direct sense more intrinsic in describing the maximum growth potential of OHO with respect to the physiological state of the bacterial cell. Even if it is not based on growth by cell multiplication, it gives a value for the instantaneous increase of the metabolism after excess substrate supply and therefore is closely associated with actual growth potential of a bacterial cell.

4. Experimental assessment of the degradation of “unbiodegradable” organic solids in activated sludge

4.1 Introduction

The prediction of sludge production and oxygen consumption in water resource recovery facilities (WRRFs) is a main objective of structured mathematical activated sludge models (ASMs) (Henze et al., 2000). However, sludge production is not only the result of solids accumulation and microbial growth on soluble substrate. It is also strongly influenced by concomitant aerobic biodegradation of organic solids. To elucidate the magnitude and characteristics of biodegradation in activated sludge, this paper deals with the experimental assessment of the degradation rate of formerly defined “unbiodegradable” organic solids in activated sludge.

In practice the total mass of organic compounds in activated sludge mixed liquor suspended solids (MLSS) is measured as VSS. However, to make this measurement usable for mass balances in activated sludge modelling the VSS is converted into Chemical Oxygen Demand (COD) units using Eq. 4.1:

$$X_{ORG} = VSS \cdot i_{cv} \quad \text{(mg COD/l)} \qquad \text{Eq. 4.1}$$

where X_{ORG} (in mg COD/l) represents particulate organic material and i_{cv} (in mg COD/mg VSS) is the COD content of the organic material.

Organic material in activated sludge models is regarded to be either biodegradable (X_{DEG}) or unbiodegradable (X_U) (see Figure 4.1 column A).

$$X_{ORG} = X_{DEG} + X_U \quad \text{(mg COD/l)} \qquad \text{Eq. 4.2}$$

The exponential decrease of X_{DEG} during aerobic digestion batch experiments means that biodegradation of X_{DEG} follows first order reaction kinetics (Metcalf and Eddy, 2004). Hence X_{DEG} can be regarded as a homogenous substrate even if biologically X_{DEG} is the sum of many different organic substances like cell plasma, cell membrane, cell internal stored material like PHA or glycogen and extracellular polymeric substances (EPS). In contrast, it is assumed that X_U is truly unbiodegradable and cannot be degraded biologically even at a slow rate.

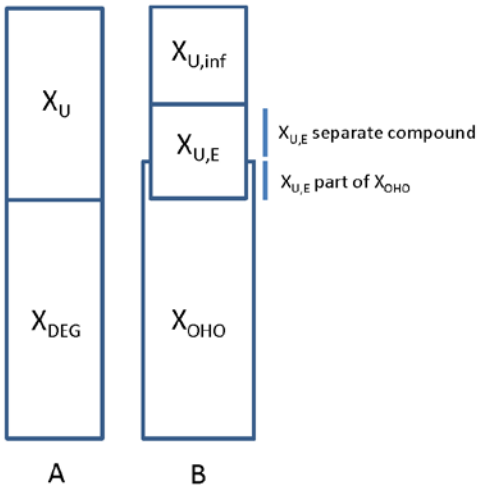

Figure 4.1: Major organic activated sludge components

However, comprehensive mechanistic activated sludge models (Marais et al., 1976) are based on a further fractionation of organic material. Ordinary heterotrophic organisms (X_{OHO}) are assumed to be the main organism group in active biomass in activated sludge and the majority of X_{OHO} is assumed to be biodegradable. But there is also an unbiodegradable portion which is termed endogenous residue $X_{U,E}$ and is determined by Eq. 4.3:

$$X_{U,E} = f_{U,E} \cdot X_{OHO} \quad \text{(mg COD/l)} \quad \text{Eq. 4.3}$$

where $f_{U,E}$ is the endogenous residue fraction of X_{OHO}. Consequently, X_{DEG} is the degradable subset of X_{OHO}:

$$X_{DEG} = (1 - f_{U,E}) \cdot X_{OHO} \quad \text{(mg COD/l)} \quad \text{Eq. 4.4}$$

The ultimate biodegradable fraction (f_{DEG}) of X_{ORG} is determined with Eq. 4.5:

$$f_{DEG} = \frac{(1 - f_{U,E}) \cdot X_{OHO}}{X_{ORG}} \quad \text{Eq. 4.5}$$

The endogenous residue fraction $f_{U,E}$ of X_{OHO} is assumed to be constant within typical municipal sludge and its value in the literature ranges from 0.15 - 0.23 (Ramdani et al., 2010). The magnitude and availability of $X_{U,E}$ in the MLSS (see Figure 4.1 column B) is mainly determined by the degradation of $X_{OHO,}$ the so-called decay process which includes death, lysis and predation by active biomass (van Loosdrecht et al.,

1999). The decay of X_{OHO} is characterised by a constant first order rate parameter (b_{OHO})(Henze et a., 1987; Gujer et al., 1999).

It is notable that the majority of the degradable material in the MLSS of an activated sludge process is associated with X_{OHO}, as the biodegradable organic material from the wastewater influent ($X_{DEG,inf}$) is assumed to be a very small fraction in activated sludge MLSS at a sludge retention time (SRT) of more than 3 days.

Another significant component of the activated sludge MLSS is the accumulated unbiodegradable solids fraction ($X_{U,inf}$) that entered the system in the influent. These solids accumulate in the mixed liquor depending on the influent loading and SRT of the system. Both fractions, the unbiodegradable endogenous residue $X_{U,E}$ and the influent unbiodegradable organic solids $X_{U,inf}$ contribute to the unbiodegradable activated sludge component $X_{U.}$

$$X_U = X_{U,E} + X_{U,inf} \quad \text{(mg COD/l)} \quad \text{Eq. 4.6}$$

Considering these three activated sludge components X_{ORG} is given by Eq. 4.7 and visualised in Fig 1 column B:

$$X_{ORG} = X_{OHO} + X_{U,E} + X_{U,inf} \quad \text{(mg COD/l)} \quad \text{Eq. 4.7}$$

The existing ASM framework is based on experimental research within system boundaries between SRTs of 3 and 20 days. In this range, sludge production predictions are quite accurate (Henze et al., 2000, Menniti et al., 2012). However, outside of that SRT range (i.e. in MBR, OSA and Cannibal systems) there seem to be deficiencies in the model predictions with regard to sludge production. In particular the phenomenon of “zero sludge production” as reported in Rosenberger et al. (2002), Pollice et al. (2004) and Laera et al. (2005) indicates that the degradation of organic material obviously cannot be explained with a single decay process. Therefore, recent research has focused on degradation processes in activated sludge and has addressed two basic assumptions of activated sludge modelling that have survived for more than four decades.

The first assumption is the unbiodegradable characteristics of X_U. Nowak et al. (1999) observed a mismatch between full-scale and pilot scale sludge production

measurements with simulation results using ASM1 and proposed a degradation process for “very slowly degradable organic material”. In a similar way Lubello et al. (2009) found an over-prediction of sludge production with ASM1 running pilot scale MBRs and introduced a hydrolysis process for the unbiodegradable particulate organics. First order kinetics was used and the rate constant q_U was determined in a range of 0.012 to 0.014 d^{-1}. Spérandio et al. (2013), using 30 references from long SRT systems, showed that the sludge production in these systems could be explained if a first order degradation rate of 0.007 d^{-1} for X_U was applied. This is in agreement with Ramdani et al. (2012) who found a range for q_U of 0.006 to 0.007 d^{-1} using pilot-scale MBR systems fed only soluble COD. Interestingly Jones et al. (2007) showed in long term anaerobic batch experiments that X_U is further degradable even in an anaerobic environment with a first order degradation rate of 0.0075 d^{-1}. The research so far does not indicate whether the process of degradation depends on the SRT of the system directly and therefore it is not yet clear when the degradation of X_U starts to become significant and detectable. Except for the Ramdani et al. (2012) study, there is little information about the specific characteristics of X_U and no information about the quality of the degradable fraction of X_U. Recently Ruiken et al. (2013) found that cellulosic material from toilet paper is a significant contributor to the particulate material in activated sludge and is partially biodegradable.

However, the obtained rates for q_U are so small that the data used for comparative prediction has to be very accurate for the validation of the model. In particular the experimental determination of the X_{OHO} degradation rate as a constant parameter with a value of 0.24 d^{-1} (Guyer et al. 1999, Fenu et al. 2010, Ramdani et al. 2010) is a crucial point for the model prediction and therefore led to challenge the second assumption.

Secondly it is assumed in the ASMs that the decay of X_{OHO} is independent of the substrate supply and therefore independent of the metabolic activity and the SRT of an activated sludge system, respectively. In a previous study (Friedrich and Takács, 2013) it was shown that the decay rate of X_{OHO} decreases with decreasing activity of X_{OHO} expressed as the specific initial oxygen uptake rate with respect to X_{ORG}. The decay rate b_{OHO} was derived from an OUR profile of an aerobic digestion batch

experiment. The procedure was based on the exclusion of an initial high OUR decrease due to the consumption of stored organic material X_{STOR}.

The first assumption that X_U is unbiodegradable, led to the VSS based method for the determination of b_{OHO} using aerobic digestion batch experiments (Ramdani et al., 2010). It was suggested that the VSS degradation profile ends at the truly unbiodegradable VSS_U. This suggestion is crucial, because if VSS_U is degradable even at a low rate (Figure 4.2 (a)) or the VSS_U detection is inaccurate (Figure 4.2 (b)), the decay rate b_{OHO} would be significantly lower.

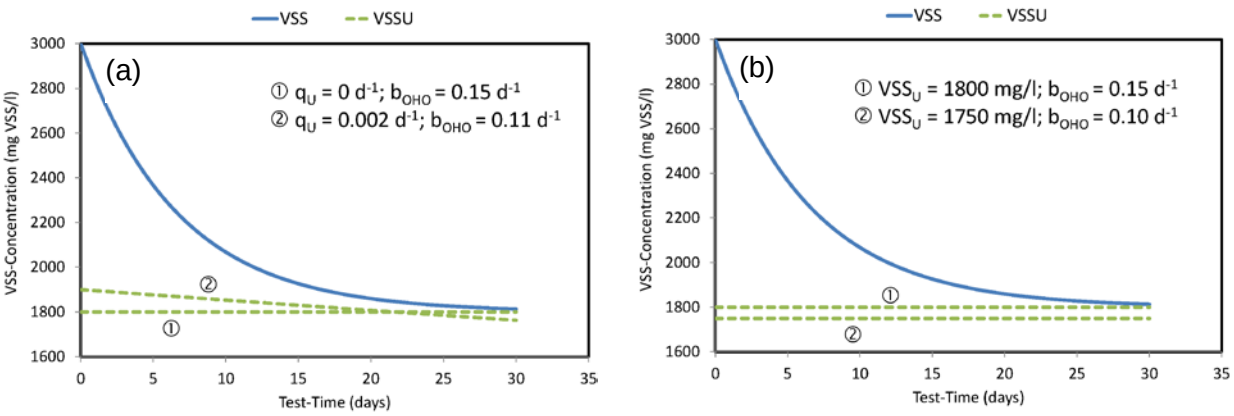

Figure 4.2: (a) b_{OHO} for decreasing VSS_U and (b) b_{OHO} for a lower VSS_U estimate

However, if the VSS degradation profile is uncertain for the determination of the degradable fraction of the MLSS in the course of an aerobic digestion batch experiment, this information can be provided by a simultaneously recorded endogenous OUR profile. The VSS measurement represents both the biodegradable and the unbiodegradable VSS fraction. In contrast, the integral of the OUR over the course of aerobic degradation stands only for the biodegraded VSS in COD units.

Therefore it is promising to combine the VSS and OUR measurements of an aerobic digestion batch experiment for the extraction of the unbiodegradable organic fraction of the MLSS and to investigate whether this fraction is constant in the course of this experiment or not.

4.2 Material and Methods

4.2.1 Origin and characterisation of activated sludge

The activated sludge for the aerobic digestion batch experiment was drawn from the return sludge of six different full scale activated sludge WRRFs ranging from 15.000 to 600.000 population equivalents (PE) situated in the Northeast of Germany. The characteristic properties are summarized in Table 4.1.

Table 4.1: Characteristics of activated sludge used in aerobic digestion batch experiments

Test	TSS	VSS	VSS/TSS	OUR(0)	spOUR(0)
	mg/l	mg/l	%	mg O_2/(l*h)	mg O_2/(g VSS*h)
A	4310	2830	68	13.8	3.2
B	4140	3020	74	20.3	4.9
C	3940	3040	77	14.5	3.7
D	3320	2663	80	25.8	7.8
E	4040	2810	69	20.4	5.0
F	3950	3060	77	17.5	4.4

Due to higher accuracy of the OUR measurements in comparison to the available data for SRT calculations, the initial specific OUR in mg O_2/(g VSS*d) of the activated sludge sample rather than the SRT was used to describe the loading state of the particular activated sludge.

With the exception of the sludge from plant C and D the WRRFs are equipped with a primary settler. The sludge samples from plants D and E were strongly influenced by industrial coffee processing wastewater. Before the experiment the sludge samples were diluted with effluent. The time from sampling to the start of the experiment was not longer than 1.5 hours.

4.2.2 Experimental setup and analytical methods

The experimental setup comprised an OUR reactor and VSS reactor (see Figure 4.3). As the activated sludge in the 2.5 l reactor of the respirometer (OUR reactor) had to remain closed to the surrounding atmosphere, it was not possible to take VSS samples from this reactor without establishing an open water surface. For this reason an open VSS sampling reactor (VSS reactor) with a volume of 15 l was simultaneously and identically operated to the OUR reactor. The VSS was measured in the first week every day and afterwards at increasing intervals of 2 to 5 days. The OUR value that belonged to a VSS measurement was taken from the computed and logged OUR measurements of the respirometer. At the end of the experiment the VSS in the two parallel reactors was „similar“, so identical degradation was assumed.

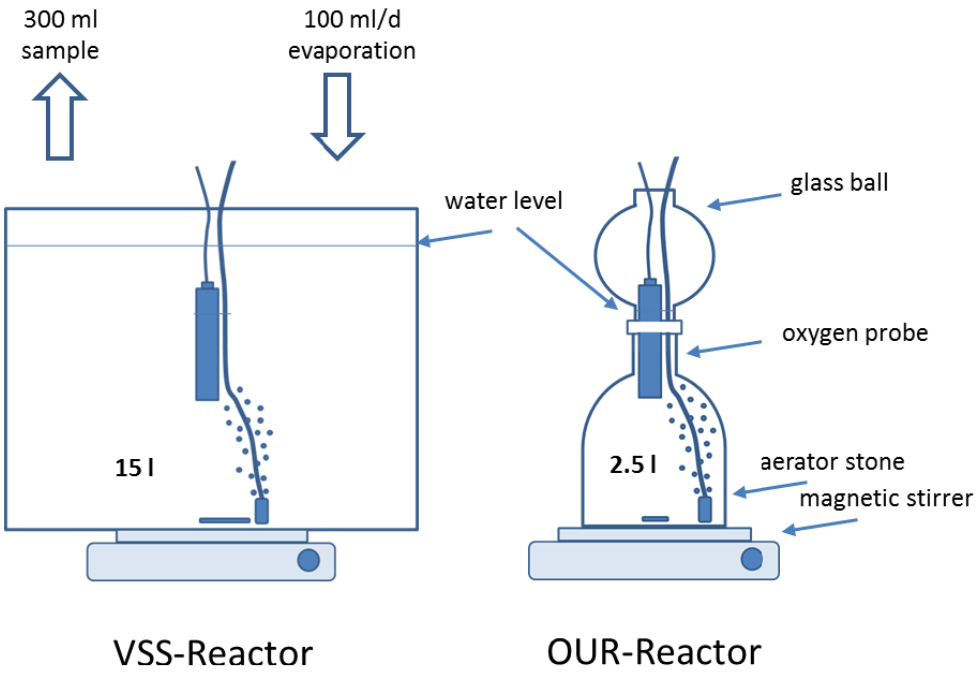

Figure 4.3: Experimental setup

The dissolved oxygen (DO) concentration in the OUR reactor as well as in the VSS reactor was operated between 4 and 6 mg O_2/l. The reactor temperature was controlled by controlling the room temperature to 20°C.

To check the quality of the experiment a COD balance was performed using the following relationship:

COD-Balance = $COD_{start} - (COD_{end} + COD_{loss} + \text{Integral } OUR_e + (NO_3\text{-}N_{end} - NO_3\text{-}N_{start})*4.57)$ Eq. 4.8

COD_{start} and COD_{end} are the COD concentrations of the activated sludge at the beginning and the end of the batch experiment, respectively. OUR_e is the endogenous oxygen uptake rate that was recorded throughout the experiment. The COD loss is obtained by collecting the total suspended solids (TSS) that was sticking to the sidewalls of the glass ball on top of the OUR reactor. On average this loss was 2% of the initial TSS. $NO_3\text{-}N_{start}$ and $NO_3\text{-}N_{end}$ are the nitrate concentration at the beginning and at the end of the batch experiment, respectively. Due to anoxic sample transportation the nitrate in the sample at the beginning of the experiment was zero. 4.57 is the electron equivalence of the NH_3 to NO_3^- conversion in nitrification.

Further details on the operation of the respirometer as well as the execution of the aerobic digestion batch experiment and the analytical methods are described in a previous research (Friedrich and Takács, 2013)

4.2.3 Method for the estimation of X_U

For the assessment of X_U, the OUR and the VSS measurements had to be combined as indicated above. As Friedrich and Takács (2013) suggested, the OUR profile of the aerobic digestion batch experiments can be modelled with Eq. 4.9:

$$OUR(t) = OUR_{STOR}(t) + OUR_{OHO}(t) + OUR_{NO3}(t) \quad (mg\ O_2/(l*h)) \quad \text{Eq. 4.9}$$

OUR_{STOR} was observed at the beginning of the aerobic digestion batch experiment with a degradation time of 1 to 5 days. Friedrich and Takács (2013) assumed that this activity was due to the degradation of stored organic material X_{STOR}. The reaction rate (q_{STOR}) of this process was comparatively high (usually > 1.0 d^{-1}) and the reaction was modelled with first order reaction kinetics according to Eq. 4.10 as a simultaneous reaction to the degradation of X_{OHO}:

$$OUR_{STOR}(t) = q_{STOR} \cdot X_{STOR}(0) \cdot e^{-q_{STOR} \cdot t} \quad (mg\ O_2/(l*h)) \quad \text{Eq. 4.10}$$

OUR_{OHO} represents the respiration rate due to the degradation of X_{OHO} and therefore corresponds to the actual decay process. The decay rate b_{OHO} is relatively low in comparison to q_{STOR}. The decay process is modelled with first order reaction kinetics according to Eq. 4.11:

$$OUR_{OHO}(t) = (1 - f_{U,E}) \cdot X_{OHO}(0) \cdot b_{OHO} \cdot e^{-b_{OHO} \cdot t} \quad \text{(mg } O_2\text{/(l*h))} \quad \text{Eq. 4.11}$$

As no nitrification inhibitor was used in any of the experiments, OUR_{NO3} due to the oxidation of the nitrogen fraction of the degraded biomass (f_N) to nitrate by nitrifiers had to be accounted for. This is modelled with Eq. 4.12.

$$OUR_{NO3}(t) = 4.57 \cdot f_N \cdot (1 - f_{U,E}) \cdot b_{OHO} \cdot X_{OHO}(0) \cdot e^{-b_{OHO} \cdot t} \quad \text{(mg } O_2\text{/(l*h))} \quad \text{Eq. 4.12}$$

The combination of Eq. 4.10 to 4.12 yields:

$$OUR(t) = q_{STOR} \cdot X_{STOR}(0) \cdot e^{-q_{STOR} \cdot t} + b_{OHO} \cdot (1 + 4.57 \cdot f_{N)} \cdot (1 - f_{U,E}) \cdot X_{OHO}(0) \cdot e^{-b_{OHO} \cdot t}$$

(mg O_2/(l*h)) Eq. 4.13

Rearranging Eq. 4.13 results an expression for the degradable fraction of the ordinary heterotrophic biomass X_{OHO}:

$$\frac{OUR(t) - q_{STOR} \cdot X_{STOR}(0) \cdot e^{-q_{STOR} \cdot t}}{b_{OHO} \cdot (1 + 4.57 \cdot f_N)} = (1 - f_{U,E}) \cdot X_{OHO}(0) \cdot e^{-b_{OHO} \cdot t} \quad \text{(mg COD/l)} \quad \text{Eq. 4.14}$$

There is a consensus that X_{OHO} dominates the degradable fraction of X_{ORG} and is therefore regarded to represent X_{DEG} according to Eq. 4.2 in terms of X_{ORG} - X_U.

$$\frac{OUR(t) - q_{STOR} \cdot X_{STOR}(0) \cdot e^{-q_{STOR} \cdot t}}{b_{OHO} \cdot (1 + 4.57 \cdot f_N)} = X_{DEG}(t) = X_{ORG}(t) - X_U(t) \quad \text{(mg COD/l)} \quad \text{Eq. 4.15}$$

Rearranging Eq. 4.15 leads to an equation that expresses X_U by parameters that nearly all can be derived directly from the aerobic digestion batch experiment.

$$X_U(t) = X_{ORG}(t) - \frac{OUR(t) - q_{STOR} \cdot X_{STOR}(0) \cdot e^{-q_{STOR} \cdot t}}{b_{OHO} \cdot (1 + 4.57 \cdot f_N)} \quad \text{(mg COD/l)} \quad \text{Eq. 4.16}$$

The OUR(t) is measured directly. The heterotrophic decay rate b_{OHO}, the degradation rate of stored organic material q_{STOR} as well as the fraction of stored organic material

$X_{STOR}(0)$ are derived from the respirogram of the first 5 days by nonlinear regression parameter estimation.

All the nitrogen that is released in the course of biodegradation of X_{DEG} will be nitrified during the aerobic digestion batch experiment. Therefore the nitrogen fraction of the degraded biomass can be measured by relating the nitrate nitrogen at the end of the experiment to the degraded VSS in terms of COD. However, it should be noted that this is a rough assumption as X_{DEG} is the sum of different degradable compounds and it is likely that the nitrogen content of these different compounds might be different, too (Lee et al., 2003).

To determine X_{ORG} the VSS is measured. However, Eq. 4.16 cannot be used directly, as X_{ORG} consists of at least two different fractions X_{DEG} and X_U, respectively. The COD content of these fractions is likely to be different from each other (Spérandio et al., 2013). With respect to this experiment the COD content for X_{DEG} ($i_{CV,DEG}$) was measured directly by relating the integral of OUR to the degraded VSS over the time of the experiment. At the end of the experiment $i_{CV,U}$ can be measured directly using standard methods for the COD and VSS analysis. In this study the COD of the MLSS was not measured. A value of $i_{CV,U}$ = 1.55 mg COD/mg VSS as the most likely value based on literature was used (Spérandio et al., 2013).

The following procedure for the assessment of $X_U(t)$, $X_{DEG}(t)$ and $X_{ORG}(t)$ was applied:

1. Calculation of $X_{DEG}(t)$ with Eq. 4.15

2. Calculation of $VSS_{DEG}(t)$ by:

$$VSS_{DEG}(t) = \frac{X_{DEG}(t)}{i_{CV,DEG}} \qquad \text{(mg VSS/l)} \qquad \text{Eq. 4.17}$$

3. Calculation of $VSS_U(t)$ by:

$$VSS_U(t) = VSS(t) - VSS_{DEG}(t) \qquad \text{(mg VSS/l)} \qquad \text{Eq. 4.18}$$

4. Calculation of $X_U(t)$ by:

$$X_U(t) = VSS_U(t) \cdot i_{CV,U} \qquad \text{(mg COD/l)} \qquad \text{Eq. 4.19}$$

Combining Eq. 4.15 to 4.19 yields an expression for the direct determination of $X_U(t)$:

$$X_U(t) = \left[VSS(t) - \frac{OUR(t) - q_{STOR} \cdot X_{STOR}(0) \cdot e^{-q_{STOR} \cdot t}}{i_{CV,DEG} \cdot [b_{OHO} \cdot (1 + 4.57 \cdot f_N)]} \right] \cdot i_{CV,U} \quad \text{(mg COD/l)} \quad \text{Eq. 4.20}$$

In comparison to an integrated modelling approach for $X_U(t)$ estimation, this procedure has the potential to identify $X_U(t)$ in the course of an aerobic digestion batch experiment with a limited number of assumptions.

However, this approach does not supply information about the build-up of $X_{U,E}$ within the degradation time of the experiment, because it considers the total $X_{U,E}$ inside and outside of the active biomass from the beginning. ASMs simulate $X_{U,E}$ after release from active biomass. The value of $X_U(t)$ in this work is basically the result of the balance of VSS(t) and OUR(t) measurements.

4.3 Results and Discussion

4.3.1 Characteristics of respirograms

The respirograms of the aerobic digestion batch experiments are displayed in Figure 4.4 and their estimated parameters are summarized in Table 4.2. The test time was between 22 and 76 days and therefore sufficiently long to detect the initial fast OUR decline that is associated with the degradation of stored material X_{STOR} and the subsequent exponential OUR decrease due to the degradation of X_{OHO} only.

The COD balance of the experiments was in the range of 99.5 to 103.7 % and the reliability of the test data can be assumed as good.

Sludge A had the highest degradation rate q_{STOR} but the lowest fraction of stored material X_{STOR}. The decay rate b_{OHO} as well as the $OUR(0)_{OHO}$ were moderate, so that the sludge could be regarded as low loaded. The SRT of this sludge was between 20 and 25 days.

Table 4.2: Results of the aerobic digestion batch experiments

Test	Test-Time	COD-Bal.	f_N	$i_{cv,DEG}$	q_{STOR}	X_{STOR}	b_{OHO}	X_{OHO}	$OUR_e(0)$	$OUR_{OHO}(0)$	f_{DEG}
	d	%	g N/g COD	g COD/g VSS	1/d	mg COD/l	1/d	mg COD/l	mg O_2/(l*h)	mg O_2/(l*h)	%
A	49	101.9	0.050	1.45	2.09	39	0.100	2509	13.8	8.3	47
B	31	100.7	0.059	1.45	1.00	100	0.129	2650	20.3	11.4	47
C	30	103.7	0.064	1.47	1.00	172	0.077	2260	14.5	5.8	39
D	42	101.3	0.058	1.42	1.00	330	0.100	2700	25.8	9.0	55
E	76	101.2	0.048	1.43	1.90	130	0.093	2650	20.4	8.2	51
F	22	99.5	0.051	1.40	0.85	180	0.094	2719	17.5	8.5	48

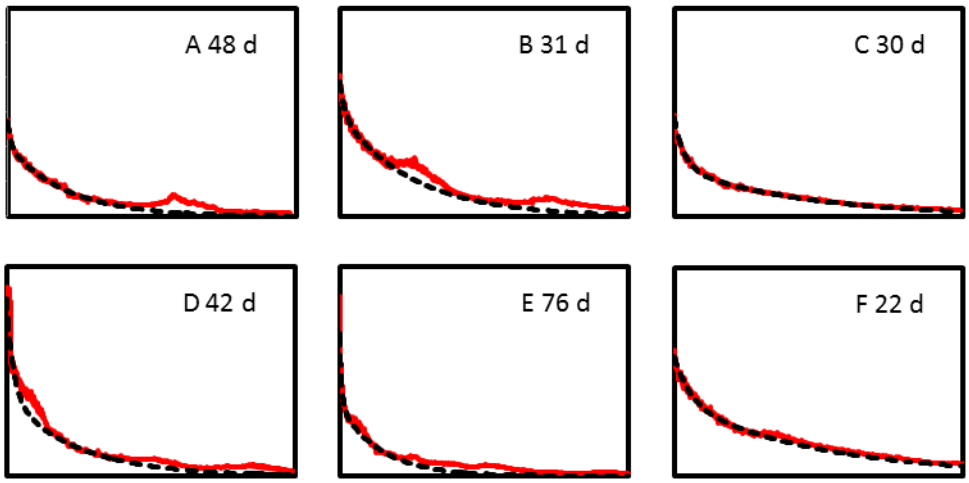

Figure 4.4: Respirograms of aerobic digestion batch tests with OUR measured (solid line) and OUR modelled (dashed line); Time = Duration of experiment; Maximum of Y-axis is OUR = 30 mg O_2/(l*h)

Sludge B had the highest decay rate b_{OHO} and the highest $OUR(0)_{OHO}$. The SRT was in a range of 10 – 12 days, so that this sludge could be regarded as comparatively high loaded. However, its X_{OHO} fraction is very close to the lower loaded sludge A.

Sludge C in contrast to sludge B had the lowest decay rate and the lowest $OUR(0)_{OHO}$. From the operators' reports it was estimated that the SRT was in the range of 70 to 80 days. Even if f_{DEG} is comparatively low, it is still close to that of sludge A and B taking the high SRT into account. The N content of sludge C in comparison to the other types of sludge is rather high. This is notable because sludge C was already aerobically digested to a very large extent. However it should be mentioned that approximately 80% of the COD load to this plant originates from slaughter house processing wastewater.

Sludge samples D and E were from different plants, but both were fed more or less regularly with coffee processing wastewater. They showed a high OUR(0) that was not consistent with their decay rates which were moderately low. This discrepancy made it hard to find an estimate for X_{OHO}.

Sludge F was again a moderately loaded sludge with an SRT of at least 15 days. Its characteristics were similar to sludge A, but had a slightly higher estimated X_{OHO} concentration.

4.3.2 Biomass concentration profiles

The results of the procedure to determine X_U, X_{DEG} and X_{ORG} are summarized in Table 4.3 and displayed as biomass concentration profiles for the examples of test D and E in Figure 4.5 (a) and (b), respectively.

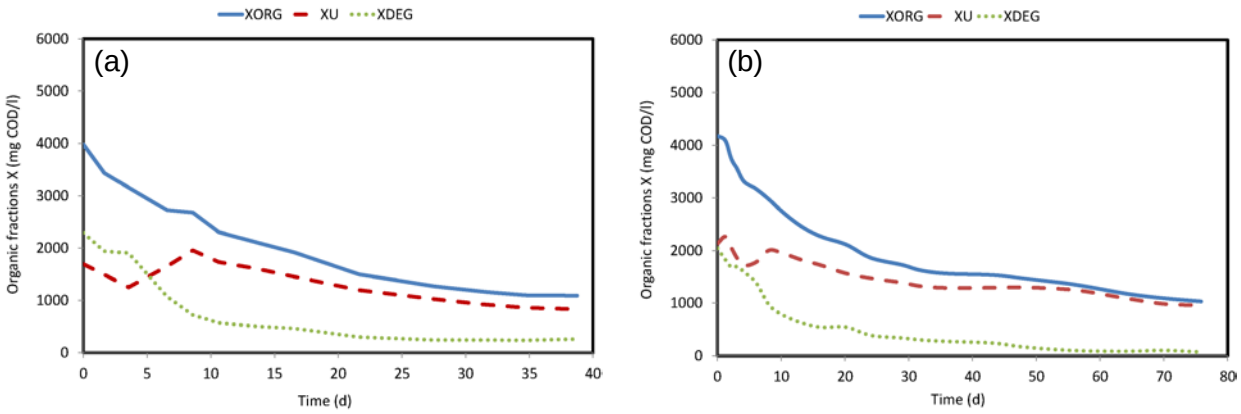

Figure 4.5: (a) Profile of organic fractions test D and (b) test E

In all experiments X_U at the end of the experiment was smaller than the initial $X_U(0)$ concentration. But the development of the $X_U(t)$ curve is not homogeneous and can be divided in two sections.

Table 4.3: Initial biomass concentration and degradation rate of X_U

	Initial concentration			X_U decrease		
Test	$X_{ORG}(0)$	$X_U(0)$	$X_{DEG}(0)$	from	q_U	R^2
	mg COD/l	mg COD/l	mg COD/l	d	d^{-1}	-
A	4246	2210	2036	18	0.011	0.685
B	4518	2153	2365	7	0.008	0.780
C	4615	2838	1778	14	0.006	0.947
D	3917	1626	2291	9	0.029	0.982
E	4177	2045	2131	10	0.009	0.959
F	4495	2179	2316	4	0.015	0.922

The first section lasts for 4 to 18 d, depending on the experiment. During this time X_U is either increasing or randomly going up and down. It is believed that this behaviour

does not reflect reality in terms of the true variation in $X_U(t)$. A factor that cannot be evaluated with sufficient certainty is the respiratory activity of protozoa. For instance in test B a mass development of the rotifer Lecane was observed and could be directly related to the first irregular OUR increase, but an associated higher VSS decrease could not be measured. Looking at test D and E it is also possible that a substrate fraction that was not included in the model of Eq. 4.9 disturbed the match of VSS and OUR within the applied approach. Furthermore it is possible that the formation of soluble microbial products (SMP) as described in the literature (Xie et al. 2012, Xie et al. 2013) led to a reduction of VSS without concomitant oxygen consumption. However, the magnitude of this phenomenon seems to be below the accuracy of the TSS measurements and not likely to occur in the course of a degradation experiment as conducted in this study. Alternatively, inadequate assumptions like a constant $i_{CV,DEG}$ and a constant f_N could lead to non-homogenous behaviour of the X_U graph evolution.

The second section showed a decrease of X_U. It is arguable whether the degradation characteristics of X_U follows first order reaction kinetics, because it is unlikely that X_U will be sufficiently homogenous. However, this simplest approach provides an opportunity to compare the results of this study with the work of other researchers (Spérandio et al. 2013; Ramdani et al. 2012; Lubello et al. 2009).

The first order rate parameter q_U was determined by linearization of X_U during the second section in a range of 0.006 d^{-1} to 0.029 d^{-1} (see Table 4.3). These results are in the order of magnitude as reported in the literature and confirm the suggestion of Nowak et al. (1999) and the results of Spérandio et al. (2013) and others to introduce a degradation process into aerobic activated sludge models for the degradation of X_U. The rather low R^2 values (especially for tests A and B) show that X_U is either not sufficiently homogenous or that microbial activity changed due to adaption of active biomass to the advancing starvation. This is illustrated very well in the respirogram of test E in Figure 4.5 (b) where a slight OUR increase and subsequent decrease after more than 40 days of digestion time points to the completion of the degradation of a certain part of X_U which obviously leads to limited microbial growth. The lowest X_U degradation rate was observed in test C. The degradation kinetics of X_U of this sludge could be measured as a true exponential OUR decrease and consequently R^2 for linearization of X_U was relatively high. This is important to note because sludge C

was already aerobically stabilized to a very large extent. Possibly at such high SRTs X_U with regard to degradability tends to become homogenous.

4.4 Conclusions

The VSS of activated sludge includes a biodegradable fraction X_{DEG} that is mainly associated with active biomass and an “unbiodegradable” fraction X_U. Furthermore the integral of the OUR is representative of the biodegradable fraction X_{DEG}.

This study has shown that it is possible to combine VSS and OUR measurements in the course of an aerobic digestion batch experiment to generate information about the degradation of X_U.

It could be shown for six different types of activated sludge that after a certain time X_U decreases until the end of the experiment. It is assumed that active biomass adapts to the severe starvation conditions with the advancing batch experiment by producing hydrolytic enzymes that support the X_U degradation.

The degradation rate q_U was estimated in the range of 0.006 d^{-1} to 0.026 d^{-1}. These experimentally produced values are consistent with the values in the literature, which were the result of simulation studies using existing ASMs.

However, more research is needed to distinguish between the degradation of “unbiodegradable” material from the influent and from bacterial decay ($X_{U,inf}$ and $X_{U,E}$). Moreover it is of interest whether there is an influence of the SRT on the degradation characteristic of X_U.

5. General Conclusions

5.1 Summary

Decay and growth of bacterial cell mass in activated sludge are two processes that are modelled traditionally with a constant growth kinetics approach, which is based on a fixed mean value for the maximum specific growth rate μ_{max} and for the endogenous decay rate b_e. Moreover the degradable fraction of VSS in activated sludge is regarded to be a homogenous fraction associated with active biomass. For a long time the rest of the VSS was assumed to be unbiodegradable. This modelling approach does not recognise the ability of microorganisms to adapt their physiology to environmental changes. The most dominant environmental factor is the availability of an energy rich substrate source resulting in a certain nutritional state that is induced by the system SRT.

In this thesis it was shown that constant growth kinetics as well as dealing with an unbiodegradable organic fraction in activated sludge cannot account for a number of observations made in aerobic degradation and growth experiments.

In particular to explain the results of aerobic degradation batch experiments the decay process had to be modelled with two degradation processes instead of one. In a rapid first process a small portion of cell constituents which might be stored material (X_{STOR}) is degraded. Notably 2 - 5 % of X_{STOR} accounts for 50% of the initial OUR. The second process is thought to be the actual decay process and runs simultaneously to X_{STOR} degradation.

By modelling the decay with two processes, the value of the decay rate parameter becomes smaller than widely used in activated sludge modelling. However, this new approach generates a more intrinsic decay rate parameter that is not constant, but variable and highly adaptive to initial respirometric activity of the activated sludge and therefore adaptive to the SRT. That means the decay rate is low for an activated sludge from a high SRT system and high for an activated sludge of a low SRT system.

Due to the adaptive decay rate the active fraction in activated sludge is more balanced then predicted with a constant decay rate approach.

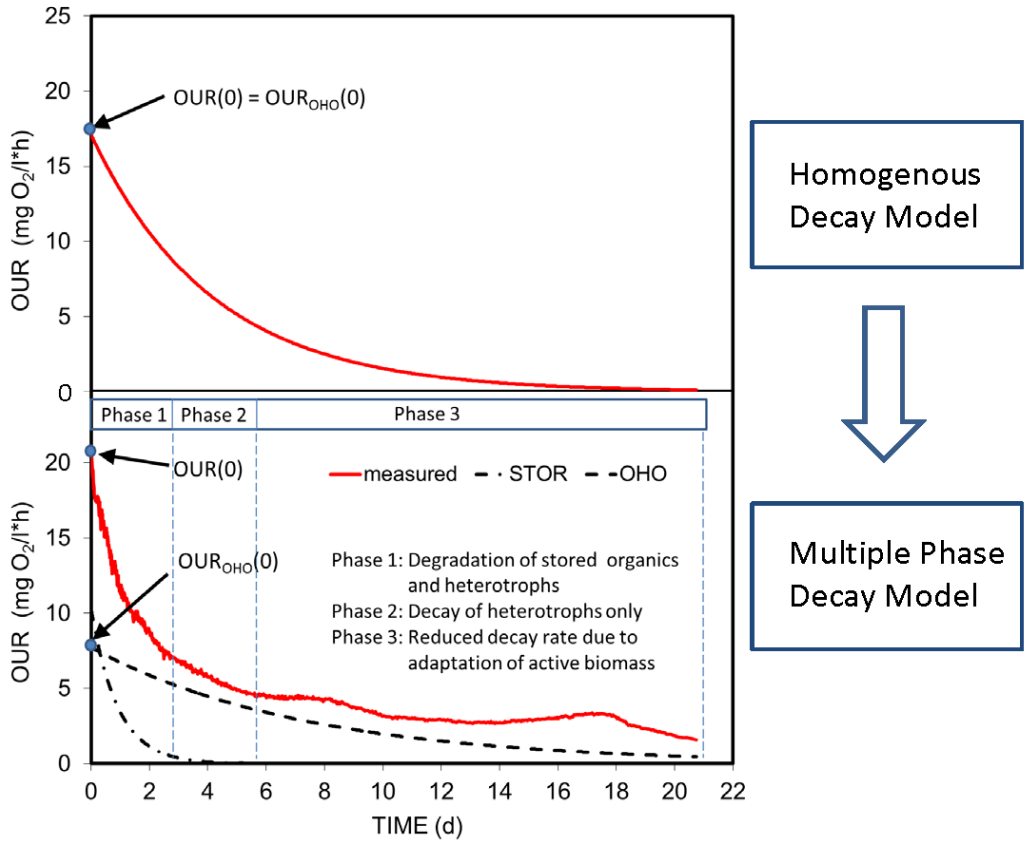

Figure 5.1: Graphical abstract for a multiple phase decay model

Furthermore by combining degradation and growth experiments it was found that there is a strong correlation of the decay rate b_e and the specific maximum growth rate μ_{max} in activated sludge. This correlation can form the basis for a new variable, adaptive growth kinetic approach for activated sludge modelling.

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.

If the value of μ_{max} is not constant but rather the result of an adaptation process driven by the nutritional state of the environment as represented by the systems SRT 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.

Transferring the characteristic of the aerobically degraded sludge (OUR_e, b_e) into an environment of excess substrate supply (OUR_{max}) leads to an equation which can be introduced to describe the maximum specific growth rate μ_{max} mainly with the ratio OUR_{max}/OUR_e and the decay rate. This is of advantage because in former approaches it was necessary to use a model to calculate the concentration of OHO for μ_{max} determination.

Finally, determining the decay rate by observing the characteristic VSS and OUR decrease in an aerobic degradation experiment, it became apparent, that both parameters did not yield the same decay rate. Comparing both the VSS and the OUR profile a procedure was developed to evaluate the decrease of the so-called unbiodegradable organic fraction in activated sludge (X_U). The measured degradation rate of X_U was in the range predicted in simulation studies.

5.2 Perspectives

As a consequence of the presented work it would be of tremendous benefit to develop an activated sludge model with an adaptive growth kinetics approach. This model would be more universal with respect to extreme SRT systems and it would predict a more balanced active biomass fraction over a wide range of SRTs.

However, it seems to be necessary to improve the experimental basis in particular for lower SRT systems regarding adaptive decay and specific maximum growth rates.

To find a comprehensive decay model it is of high importance to describe the first rapid degradation process more accurately. It is of interest whether stored material or other cell constituents are subjected to decay. Even if the biomass fraction degraded in this process is small, the respiratory activity is very high which gives this process an importance that should not be underestimated.

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