Modelling particle degradation and intermediate dynamics in a dispersed activated sludge microcosm

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Abstract

Municipal wastewater consists of a large fraction of particulate organic matter. During biological wastewater treatment these particles undergo extracellular depolymerisation before products are taken up by bacteria (MW < 0.6 kDa). Particle degradation and intermediate formation dynamics is important in process analysis of wastewater treatment as the transport regime differ. This work aims to develop a model for particle degradation that includes intermediate dynamics as observed in experimental work. A model for particle degradation including intermediate dynamics, bacterial growth and endogenous respiration is proposed. Particle hydrolysis was modelled using the particle breakup model. Depolymerisation products were separated into five different size groups: colloids; high, medium and low molecular weight (HMW, MMW and LMW) polymers; and one fraction for oligomers and monomers (S_B). Depolymerisation of colloids, HMW and MMW polymers was modelled using first order kinetics. LMW polymer degradation was modelled using Michaelis-Menten kinetics, while growth was based on traditional Monod kinetics and endogenous respiration followed ASM3. The proposed model was implemented in AQUASIM for a batch reactor system, and parameter estimation by LSE fitting to experimental data on particulate starch degradation over 117 days in a dispersed biomass microcosm was performed. Validation of the model against experimental data gave a very good fit to the PBM. The intermediate dynamics seen in the experimental data was also qualitatively demonstrated by the model, with accumulation of HMW, MMW and LMW polymers in the bulk liquid. However, the accumulation of monomers and oligomers in the bulk liquid could not be reproduced in the suspended growth model proposed. Hence, a structured biomass model (biofilm) is suggested for future work.

1. Introduction

Wastewater consists of a large fraction of particulate organic matter (POM) (Levine et al., 1991; Ravndal et al., 2018). During biological wastewater treatment these particles must undergo extracellular depolymerisation before products can be taken up by bacteria (M_W < 0.6 kDa) (Decad and Nikaido, 1976; White et al., 2012). Organic matter (OM) proceeds through a range of colloidal and polymeric intermediates during this process. The dominant mechanisms for this degradation are hydrolytic and lytic depolymerisation, and theoretically this allows for any sub-polymeric intermediates to be formed. Hence, both particle degradation and intermediate formation are important in process analysis of wastewater treatment.

Over the years, several different approaches have been proposed for modelling of particle degradation in wastewater treatment processes (Hauduc et al., 2013; Morgenroth et al., 2002; Vavilin et al., 2008). These models include one step, parallel and sequential hydrolysis, in addition to direct growth using adsorbed substrate and different types of surface related kinetics. For surface related kinetics two different models have been proposed, these are the shrinking particle model (SPM; Sanders et al., 2000) and the particle breakup model (PBM; Dimock and Morgenroth, 2006). Hydrolysis is dependent on available surface area in both models. However, in the SPM particles shrink gradually as they are degraded, thus available surface area decreases. While in the PBM particles break up as they are degraded, leading to an increase in available surface area, and therefore increasing substrate availability over time. Hence, in the PBM surface area to volume ratio is considered, whereas surface area is used in the SPM. An open question of the PBM is that an increase in particle porosity and increased particle colonization could also lead to increased substrate availability over time (Dimock and Morgenroth, 2006). Hence the same dynamics would be observed as when particles physically break up by hydrolysis.

In previous work by the authors an experimental study was performed to look at particle degradation in activated sludge microcosms (Ravndal and Kommedal, 2017). In this study starch was used as a model particle substrate. Starch is a good model substrate as it has a known structure, at the same time as much of the complexity expected for unidentified particles in wastewater are represented in starch granules. Starch is a natural component in

wastewater, and starch degrading bacteria are commonly found in activated sludge systems (Xia et al., 2008). In the experimental test starch particles were colonised by bacteria, leading to both increase in particle porosity and particle breakup (Ravndal and Kommedal, 2017). Polymeric, oligomeric and monomeric intermediates formed during particle degradation, however, not all intermediate sizes were formed to the same extent. Based on the data a conceptual model was proposed for chemical oxygen demand (COD) flow during starch depolymerisation. This conceptual model assumed the surface of the particle to be a hotspot for extracellular hydrolytic activity. The different intermediates were grouped based on size and included in the conceptual model.

In this paper the aim is to develop a mathematical model for particle degradation that includes intermediate dynamics as observed in the experimental work by Ravndal and Kommedal (2017). This is done by developing a model including intermediate dynamics, bacterial growth, and endogenous respiration, and fitting the model to the experimental data.

2. Methodology

The model was developed based on experimental data previously published in Ravndal and Kommedal (2017). In this experiment starch degradation was followed over 117 days in batch tests inoculated with flocculated and dispersed activated sludge biomass. During the experiment the following was monitored: Oxygen utilisation rate (OUR), particle number and size (volume and surface area), polymer concentration and size (molar mass), and oligomer and monomer type and concentrations.

2.1. Model development

The experimental data in Ravndal and Kommedal (2017) supported the PBM proposed by Dimock and Morgenroth (2006). Hence, this model was chosen for particle degradation in the proposed model (Tab. 1). The PBM is based on surface area to volume ratio (f_{av}). f_{av} was included as a state variable in the model and change in f_{av} was directly coupled to the hydrolysis process with the constant c_{av}. Colloids (C_B) and polymeric intermediates are formed during particle degradation. Theoretically all intermediate sizes of polymers can be formed during particle degradation, however, for simplicity the polymers in the model were grouped into three groups based on size ranges; high molecular weight (HMW, $S_{pol,HMW}$), medium molecular weight (MMW, $S_{pol,MMW}$) and low molecular weight (LMW, $S_{pol,LMW}$) polymers. Oligomers and monomers were grouped into one state variable, S_B, representing readily biodegradable substrate small enough to be taken up by bacteria.

To minimize model parametrisation, degradation of colloids, HMW and MMW polymers was implemented using first order biomass independent kinetics (Tab. 1). LMW polymer degradation was modelled using Michaelis-Menten kinetics (Tab. 1). Most Michaelis-Menten constants (K_m) for hydrolytic enzymes are high (Technical University of Braunschweig, 2022), hence, in activated sludge where enzyme concentrations are high, simplification to first order kinetics can normally be assumed. However, LMW polymers are a common product of depolymerisation of starch, colloids and larger polymeric substrates (Robyt, 2009), hence large substrate concentrations can also be achieved.

Table 1: Process matrix for starch degradation. Particle hydrolysis was based on the PBM (Dimock and Morgenroth, 2006), colloids, HMW and MMW polymer degradation followed first order biomass independent kinetics, LMW polymer degradation followed Michaelis Menten kinetics, growth was based on traditional Monod kinetics, and endogenous respiration was based on ASM3 (Henze et al., 2000). Nomenclature based on Corominas et al. (2010).

Process	S_{O2}	S_B	$S_{pol,LMW}$	$S_{pol,MMW}$	$S_{pol,HMW}$	C_B	$f_{av,X}$	X_B	X_U	X_{OHO}	Rate
Particle hydrolysis		f_{SB_X}	f_{LMW_X}	f_{MMW_X}	f_{HMW_X}	f_{CB_X}	c_{av}	-1			$q_X f_{av,X} X_B$
Colloid hydrolysis		f_{SB_C}	f_{LMW_C}	f_{MMW_C}	f_{HMW_C}	-1					$q_{C_B} C_B$
HMW polymer hydrolysis		f_{SB_HMW}	f_{LMW_HMW}	f_{MMW_HMW}	-1						$q_{Spol,HMW} S_{pol,HMW}$
MMW polymer hydrolysis		f_{SB_IMW}	f_{LMW_IMW}	-1							$q_{Spol,MMW} S_{pol,MMW}$
LMW polymer hydrolysis		1	-1								$q_{Spol,LMW} \frac{S_{pol,LMW}}{K_{m,LMW} + S_{pol,LMW}} X_{OHO}$
Growth on S_B	$-\frac{1 - Y_{OHO}}{Y_{OHO}}$	$-\frac{1}{Y_{OHO}}$								1	$\mu_{OHO,Max} \left(\frac{S_B}{K_{S,OHO} + S_B} \right) X_{OHO}$
Endogenous respiration	$-(1 - f_{XU})$								f_{XU}	-1	$b_h X_{OHO}$

In addition, enzyme affinity increases with a decreasing polymer size. Hence, Michaelis-Menten kinetics was used for LMW polymer degradation. For particle, colloid, HMW, MMW and LMW polymers smaller intermediate sizes are formed, and fractions of each intermediate formed is included in the process matrix in Tab. 1. Traditional Monod kinetics was used for growth on S_B, and endogenous respiration from ASM3 was implemented for active biomass decay (Henze et al., 2000).

2.2. Model implementation

The model was implemented in AQUASIM (Reichert, 1994) using a simple mixed reactor compartment for the test bottle liquid phase. This was coupled by a diffusive link to a mixed reactor gas phase compartment representing the headspace in the test bottles. Parameter estimation was performed by LSE fitting the model to experimental data from the dispersed activated sludge microcosms published in (Ravndal and Kommedal, 2017). The following experimental data were used: Total particle volume (X_{vol}), HMW, MMW and LMW polymer concentrations, S_B concentration, and OUR. Total particle volume was defined as the sum of starch particle volume ($X_{B,vol}$), biomass volume ($X_{OHO,vol}$) and inert particle volume ($X_{U,vol}$) (eq. 1).

$$X_{vol} = X_{B,vol} + X_{OHO,vol} + X_{U,vol} \quad (1)$$

Particle volume was related to COD concentrations using density and theoretical oxygen demand (ThOD) of the different particle types. In addition, starch particle swelling was observed in the early phase of the experiment and had to be accounted for. A swelling constant (f_{swell}) for starch particles was estimated based on measured initial volume increase. f_{swell} was set to increase linearly from 1 at time 0 to 1.53 after 5.93 d, and then kept constant throughout the experiment. Starch particle volume ($X_{B,vol}$) can be related to starch COD concentration (X_B) using eq. 2. Where ρ_{XB}, the density of potato starch granules, is $1.54^{*}10^6$ g m^{-3}, (BNID103206, Milo et al., 2010), and the ThOD of starch (fv_{XB}) is 1.19 g COD (g X_B)$^{-1}$.

$$X_{B,vol} = \frac{X_B}{fv_{XB}*\rho_{XB}} f_{swell} \quad (2)$$

Volume of microbial biomass ($X_{OHO,vol}$) was estimated based on literature data for *Escherichia coli* cells. Average density (ρ_{XOHO}) of an *E.coli* cell is 1.094 g mL^{-1} (BNID106306, Milo et al., 2010). The ThOD of bacterial biomass (fv_{XB}) is 1.42 g COD (g X_{OHO})$^{-1}$. The volume of bacterial biomass was related to biomass COD concentration (X_{OHO}) using eq. 3.

$$X_{OHO,vol} = \frac{X_{OHO}}{fv_{XOHO}*\rho_{XOHO}} \quad (3)$$

Inert particle volume ($X_{U,vol}$) was estimated with the same ThOD and density as for microbial biomass. ThOD of polymeric intermediates was the same as for starch particles. Monomer and oligomer concentrations were related to individual specific ThODs and summarised in one state variable, S_B.

3. Results and discussion

3.1 Parameter estimation

Estimated stoichiometric parameters are summarised in Tab. 2. The experimental data showed that the MMW polymer concentration started to increase at the same time as the system shifted from a starch particle dominated system to a HMW polymer dominated system (Ravndal and Kommedal, 2017). Hence, MMW polymers where most likely degradation products of HMW polymer depolymerisation, and f_{MMW_X} and f_{MMW_C} was estimated as zero (Tab. 2).

Starch granules consist of a mix of amylopectin (70-80 %) and amylose (20-30 %) (Dona et al., 2010). Amylopectin is the largest of the two polymers with a molecular weight of 10^4-10^6 kDa, it is highly branched containing 5 % α-1,6 branches and water soluble (Ball et al., 1996; Shannon et al., 2009). Amylose is a smaller polymer with molecular weight of 100-1000 kDa, it is essentially linear with less than 1 % α-1,6 branches and has variable solubility depending on branching where linear amylose is essentially insoluble in water (Ball et al., 1996; Mukerjea and Robyt, 2010). During particle degradation these polymers will be released from the starch granule. Due to the large size of amylopectin and the amylose being mostly insoluble, these polymers are expected to behave as colloids initially. Hence, f_{HMW_X} was also estimated as zero (Tab. 2).

Both the LMW polymer fraction and the grouped S_B state variable contains several known degradation products from enzymatic degradation of starch (Robyt, 2009). All hydrolysis processes therefore led to fractions of these two variables (Tab. 2). The fractions increased, the closer in size the initial variable was. Because parameter estimation in Aquasim did not ensure that the sum of all fractions was one, it was chosen to manually test different numbers for the fractions and the numbers giving the best fit with the experimental data was chosen.

Kinetic parameters for the extracellular degradation processes (Tab. 3) were estimated using LSE parameter estimation in Aquasim. The estimated hydrolysis rate constants increased with decreasing substrate size. This is expected, as faster degradation is expected for smaller substrate sizes.

Table 2: Stoichiometric parameters estimated for the starch particle degradation model.

Parameter	Definition	Value [1]	Reference
f_{CB_X}	Fraction of colloids formed in particle hydrolysis	0.45	Estimated
f_{HMW_X}	Fraction of HMW polymers formed in particle hydrolysis	0	Estimated
f_{MMW_X}	Fraction of MMW polymers formed in particle hydrolysis	0	Estimated
f_{LMW_X}	Fraction of LMW polymers formed in particle hydrolysis	0.2	Estimated
f_{SB_X}	Fraction of monomers and oligomers (S_B) formed in particle hydrolysis	0.35	Estimated
f_{HMW_C}	Fraction of HMW polymers formed in colloid hydrolysis	0.15	Estimated
f_{MMW_C}	Fraction of MMW polymers formed in colloid hydrolysis	0	Estimated
f_{LMW_C}	Fraction of LMW polymers formed in colloid hydrolysis	0.45	Estimated
f_{SB_C}	Fraction of monomers and oligomers (S_B) formed in colloid hydrolysis	0.4	Estimated
f_{MMW_HMW}	Fraction of MMW polymers formed in hydrolysis of HMW polymers	0.5	Estimated
f_{LMW_HMW}	Fraction of LMW polymers formed in hydrolysis of HMW polymers	0.25	Estimated
f_{SB_HMW}	Fraction of monomers and oligomers (S_B) formed in hydrolysis of HMW polymers	0.25	Estimated
f_{LMW_MMW}	Fraction of LMW polymers formed in hydrolysis of MMW polymers	0.75	Estimated
f_{SB_MMW}	Fraction of monomers and oligomers (S_B) formed in hydrolysis of MMW polymers	0.25	Estimated
Y_{OHO}	Aerobic yield of ordinary heterotrophic organisms	0.5	(Sykes, 1975)
f_{XU}	Production of inert (X_U) in endogenous decay	0.2	(Henze et al., 2000)

Table 3: Kinetic parameters estimated for the starch particle degradation model.

Parameter	Definition	Value	Reference
q_X	Modified hydrolysis constant for particles in PBM	$1.25^{*}10^{-9}$ m d^{-1}	Estimated
c_{av}	PBM constant relating particle breakup to hydrolysis rate	$1.54^{*}10^{5}$ m^2 g^{-1}	Estimated
q_{C_B}	Hydrolysis rate constant for colloid degradation	0.05 d^{-1}	Estimated
$q_{Spol,HMW}$	Hydrolysis rate constant for degradation of HMW polymers	0.08 d^{-1}	Estimated
$q_{Spol,MMW}$	Hydrolysis rate constant for degradation of MMW polymers	0.09 d^{-1}	Estimated
$q_{Spol,LMW}$	Hydrolysis rate constant for degradation of LMW polymers	0.3 d^{-1}	Estimated
$K_{m,LMW}$	Michaelis-Menten constant for degradation of LMW polymers	93 g COD m^{-3}	Estimated
$\mu_{OHO,Max}$	Maximum growth rate for heterotrophic organisms	8 d^{-1}	Estimated
$K_{S,OHO}$	Saturation constant for substrate S_B	4 g COD m^{-3}	Estimated
b_h	Aerobic endogenous respiration rate of X_{OHO}	0.15 d^{-1}	(Henze et al., 2000)

The proposed model contains many different stoichiometric and kinetic parameters (Tab. 2 and 3). Some of these were estimated manually, some estimated using parameter estimation in Aquasim and some are based on literature data. To test for parameter identifiability a sensitivity analysis could be done. This analysis is not included here, but it is suggested to include this in future work on an improved version of the model.

3.2 Are the model able to reproduce the experimental data?

The model output is compared to experimental data for OUR, particle volume and concentrations of different intermediates in Fig. 1 and Fig. 2. In the experiment, the OUR increased fast between day 2 and 4 before it stabilised at a level of 1.8 mg L^{-1} h^{-1} until day 36, before a steady decrease occurred (Fig. 1). The model was not able to reproduce the initial increase and the stable phase, however, the decrease after 36 days was reproduced by the model.

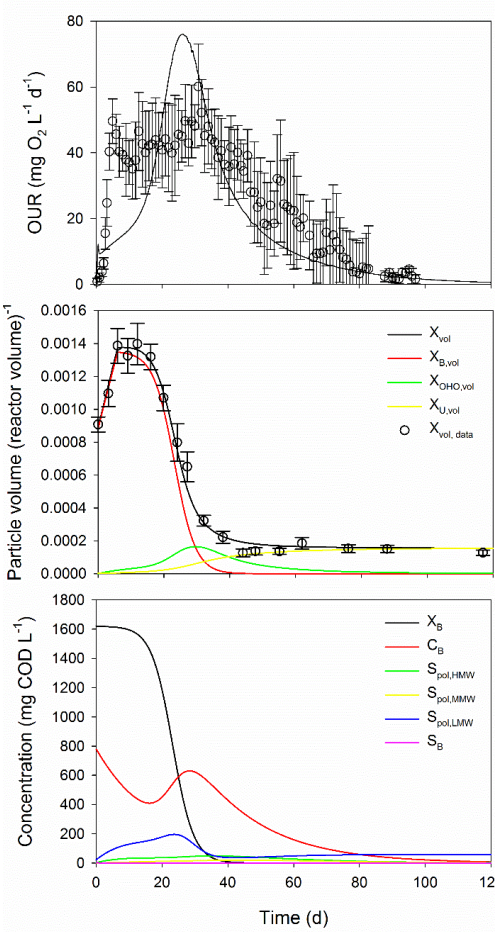

Figure 1: Experimental data (point ± standard error bars) and modelled data (lines) for OUR, particle volume and substrate concentrations in dispersed biomass tests.

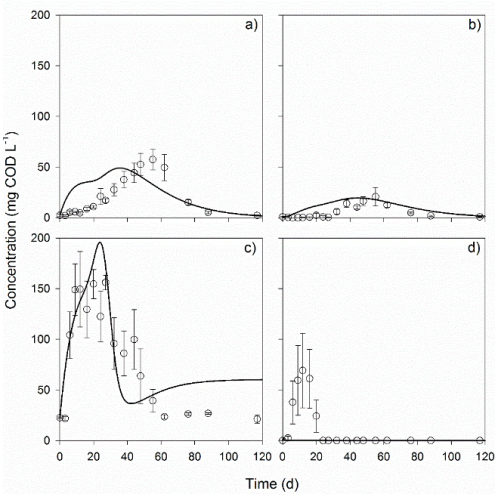

Figure 2: a) HMW polymers, b) MMW polymers, c) LMW polymers and d) monomers and oligomers (S_B). Points (± standard error bars) show experimental data for dispersed biomass tests, lines show modelled data.

Total particle volume fitted the PBM model very well, with growth and decay modelled after ASM3 (Fig. 1). This supported the conclusion in Ravndal and Kommedal (2017) of the data supporting the PBM proposed by Dimock and Morgenroth (2006). LMW, MMW and HMW polymers were produced and accumulated in the bulk liquid in the experiment (Ravndal and Kommedal, 2017). In the model the initial LMW peak had a good fit to the experimental data, while a faster accumulation of MMW and HMW polymers was seen compared to the experimental dataset (Fig. 2).

Colloids was included as a state variable in the model. However, we did not have access to detection methods covering the colloidal size range in the experiment. This made it challenging to identify all parameters in the model. As HMW polymers are in the size range immediately below colloids, a better fit of HMW polymer production would be expected if the experimental dataset had included colloids concentrations.

S_B accumulated in the bulk liquid in the experiment, however, this data was not reproduced by the model (Fig. 2). Apart from a tiny peak reaching a maximum of 3 mg L^{-1} of S_B after 0.37 d, no accumulation of S_B was seen in the modelled data. Hence, after the start up, the uptake of S_B by the biomass in the model was faster than the hydrolysis processes.

3.3 Model limitations and suggestions for improvements

The lack of S_B accumulating in the bulk liquid in the modelled data compared to the experimental data (Fig. 2) showed that the model did not account for diffusion limitations in the system. The model was a simple suspended growth model, however, the experiment showed that in reality the biomass colonized the surface of the starch particles (Ravndal and Kommedal, 2017). Hence, a small biofilm was formed by the colonizing biomass leading to diffusion limitations for any intermediate in the bulk liquid. To allow for S_B accumulation due to diffusion limitations, a heterogeneous biomass structure must be considered.

A model based on activated sludge flocs as small bio-aggreagates with diffusion limitations and sorption dynamics, would greatly improve modelling of intermediate dynamics compared to the simplified model presented in this work. Diffusion limitations and sorption dynamics would allow for accumulation of all intermediates, including monomers and oligomers, in the bulk liquid. Hence, the initial increase of monomers and oligomers as seen in the experimental data, would be present in the modelled data. The delayed accumulation of MMW and HMW polymeric intermediates in the experimental data compared to the simplified model output, would also be recreated in a bio-aggregate model. However, including these factors in the

model will increase model complexity, thus, complicate model identification and validation. Model complexity must be balanced to the need for detailed understanding of degradation dynamics and acceptable uncertainty.

To validate a bio-aggregate model, data on bio-aggregate volume, depth and surface are needed. In the experimental work used to validate the model in this paper, this data is lacking as the particle size measurements is dominated by the starch particle fraction. To further improve modelling of intermediate dynamics during particle degradation it is suggested to combine this type of experimental work with work on a bio-aggregate model.

4. Conclusions

A mathematical model for particle degradation, including intermediate dynamics, was proposed in this work.

- The PBM fitted experimental data for particulate substrate well.
- Accumulation of LMW, MMW and HMW polymeric fractions in the bulk liquid was qualitatively replicated by the model, with the initial peak of LMW polymers giving the best fit to the experimental data.
- The model was unable to replicate data showing oligomers and monomers to accumulate in the system. It is suggested to include a bio-aggregate model in further work to account for diffusion limitations in the system due to the biomass growing as a colonizing biofilm on the starch particles.

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References

Ball, S., Guan, H.P., James, M., Myers, A., Keeling, P., Mouille, G., Buleon, A., Colonna, P. and Preiss, J. (1996) 'From glycogen to amylopectin: A model for the biogenesis of the plant starch granule', *Cell*, Cell Press, 86(3), pp. 349-352. doi: 10.1016/s0092-8674(00)80107-5

Corominas, L., Rieger, L., Takács, I., Ekama, G., Hauduc, H., Vanrolleghem, P.A., Oehmen, A., Gernaey, K.V., van Loosdrecht, M.C.M. and Comeau, Y. (2010) 'New framework for standardized notation in wastewater treatment modelling', *Water Science and Technology*, IWA Publishing, 61(4), pp. 841-857. doi: 10.2166/wst.2010.912

Decad, G.M. and Nikaido, H. (1976) 'Outer membrane of gram-negative bacteria. XII. Molecular-sieving function of cell wall', *Journal of Bacteriology*, ASM Journals, 128(1), pp. 325-336. doi: 10.1128/jb.128.1.325-336.1976.

Dimock, R. and Morgenroth, E. (2006) 'The influence of particle size on microbial hydrolysis of protein particles in activated sludge', *Water Research*, Elsevier, 40(10), pp. 2064-2074. doi: 10.1016/j.watres.2006.03.011

Dona, A.C., Pages, G., Gilbert, R.G. and Kuchel, P.W. (2010) 'Digestion of starch: In vivo and in vitro kinetic models used to characterise oligosaccharide or glucose release', *Carbohydrate Polymers*, Elsevier, 80(3), pp. 599-617. doi: 10.1016/j.carbpol.2010.01.002

Hauduc, H., Rieger, L., Oehmen, A., van Loosdrecht, M.C.M., Comeau, Y., Heduit, A., Vanrolleghem, P.A. and Gillot, S. (2013) 'Critical review of activated sludge modeling: State of process knowledge, modeling concepts, and limitations', *Biotechnology and Bioengineering*, Wiley Periodicals, 110(1), pp. 24-46. doi: 10.1002/bit.24624

Henze, M., Gujer, W., Mino, T. and van Loosdrecht, M. (2000) *Activated sludge models ASM1, ASM2, ASM2d and ASM3*. IWA Publishing.

Levine, A.D., Tchobanoglous, G. and Asano, T. (1991) 'Size distributions of particulate contaminants in wastewater and their impact on treatability', *Water Research*, Elsevier, 25(8), pp. 911-922. doi: 10.1016/0043-1354(91)90138-g

Milo, R., Jorgensen, P., Moran, U., Weber, G. and Springer, M. (2010) 'BioNumbers—the database of key numbers in molecular and cell biology', *Nucleic Acids Research*, Oxford Academic, 38(suppl_1), pp. D750-D753. doi: 10.1093/nar/gkp889

Morgenroth, E., Kommedal, R. and Harremoes, P. (2002) 'Processes and modeling of hydrolysis of particulate organic matter in aerobic wastewater treatment - a review', *Water Science and Technology*, IWA Publishing, 45(6), pp. 25-40. doi: 10.2166/wst.2002.0091.

Mukerjea, R. and Robyt, J.F. (2010) 'Isolation, structure, and characterization of the putative soluble amyloses from potato, wheat, and rice starches', *Carbohydrate Research*, Elsevier, 345(3), pp. 449-451. doi: https://doi.org/10.1016/j.carres.2009.11.021

Ravndal, K.T. and Kommedal, R. (2017) 'Starch degradation and intermediate dynamics in flocculated and dispersed microcosms', *Water Science and Technology*, IWA Publishing, 76(11), pp. 2928-2940. doi: 10.2166/wst.2017.467

Ravndal, K.T., Opsahl, E., Bagi, A. and Kommedal, R. (2018) 'Wastewater characterisation by combining size fractionation, chemical composition and biodegradability', *Water Research*, Elsevier, 131, pp. 151-160. doi: https://doi.org/10.1016/j.watres.2017.12.034

Reichert, P. (1994) 'AQUASIM - a tool for simulation and data-analysis of aquatic systems', *Water Science and Technology*, IWA Publishing, 30(2), pp. 21-30. doi: 10.2166/wst.1994.0025

Robyt, J.F. (2009) Starch: Chemistry and technology. BeMiller, J. and Whistler, R. (eds), pp. 237-292, Academic Press.

Sanders, W.T.M., Geerink, M., Zeeman, G. and Lettinga, G. (2000) 'Anaerobic hydrolysis kinetics of particulate substrates', *Water Science and Technology*, IWA Publishing, 41(3), pp. 17-24. doi: 10.2166/wst.2000.0051

Shannon, J.C., Garwood, D.L. and Boyer, C.D. (2009) Starch: Chemistry and Technology. BeMiller, J. and Whistler, R. (eds), pp. 23-82, Academic Press.

Sykes, R.M. (1975) 'Theoretical Heterotrophic Yields', *Journal (Water Pollution Control Federation)*, Wiley, 47(3), pp. 591-600. http://www.jstor.org/stable/25038666.

Technical University of Braunschweig. (2022) BRENDA: The comprehensive enzyme information system, Available at: http://www.brenda-enzymes.org/index.php, (Accessed: 30.05.22)

Vavilin, V.A., Fernandez, B., Palatsi, J. and Flotats, X. (2008) 'Hydrolysis kinetics in anaerobic degradation of particulate organic material: An overview', *Waste Management*, Elsevier, 28(6), pp. 939-951. doi: 10.1016/j.wasman.2007.03.028

White, D., Drummond, J. and Fuqua, C. (2012) *The physiology and biochemistry of prokaryotes*.4th edn. Oxford university press.

Xia, Y., Kong, Y.H. and Nielsen, P.H. (2008) 'In situ detection of starch-hydrolyzing microorganisms in activated sludge', *Fems Microbiology Ecology*, Oxford Academic, 66(2), pp. 462-471. doi: 10.1111/j.1574-6941.2008.00559.x