Processes and modeling of hydrolysis of particulate organic matter in aerobic wastewater treatment – a review

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Abstract Carbon cycling and the availability of organic carbon for nutrient removal processes are in most wastewater treatment systems restricted by the rate of hydrolysis of slowly biodegradable (particulate) organic matter. To date, the mechanisms of hydrolysis are not well understood for complex substrates and mixed populations. Most mathematical models use a simple one-step process to describe hydrolysis. In this article, mechanisms of hydrolysis and mathematical models to describe these processes in wastewater treatment processes are reviewed. Experimental techniques to determine mechanisms of hydrolysis and rate constants are discussed.

Keywords Aerobic treatment; hydrolysis; modelling; municipal wastewater; particulate organic matter

Introduction

A major fraction of organic material in municipal wastewater is in the particulate form and has to be hydrolyzed before it can be taken up and be degraded by bacteria (Levine *et al*., 1985). A quantitative understanding of the availability of particulate organic matter is required to predict the overall performance of wastewater treatment plants and local oxygen demand. Nutrient removal (denitrification and biological phosphorus removal) are in many cases limited by the extent and kinetics of hydrolysis processes and particulate organic matter can be important for the selection of specific bacterial populations (Frigon *et al*., 2001). As long as wastewater treatment plants did not require denitrification or biological phosphorus removal, the ultimate fate of particulate organic matter in the wastewater was less important. Particulate organic matter attaches rapidly to the sludge flocs and is then either degraded or removed with the excess sludge. In either case, the treatment objective of removing the organic matter from the effluent is achieved. In the design of reactors in a nutrient removal treatment plant, hydrolysis rates determine the conversion of slowly biodegradable organic matter into readily biodegradable organic matter that can serve as a necessary carbon source for denitrification or biological phosphorus removal. The particulate organic fraction and associated hydrolysis rates has a direct impact on the volume of nutrient removing treatment plants. In the following review, we present an overview over particle characteristics in wastewater, mechanisms involved in hydrolysis, mathematical models to describe hydrolysis, and experimental tools to determine model parameters.

Definition of hydrolysis

Hydrolysis refers to the breakdown of organic substrate into smaller products that can subsequently be taken up and degraded by bacteria. Two types of hydrolysis can be differentiated: (a) hydrolysis of primary substrate where organic substrate present in the original wastewater is broken down; (b) hydrolysis of secondary substrate that refers to the breakdown of substrate that has been produced by the bacteria (e.g. hydrolysis of internal storage products, of substances released by the bacteria during normal metabolism, or of particles

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produced during decay of bacteria) (Bryers and Mason, 1987, van Loosdrecht and Henze, 1999). It should be noted that in ASM1 (Henze *et al.*, 1987) and ASM3 (Gujer *et al.*, 1999) the term hydrolysis is used differently. In ASM1, hydrolysis refers to the sum of primary and secondary hydrolysis while in ASM3 hydrolysis is separated from the turnover of internal products (Figure 1). This paper is focused on primary hydrolysis. The strict definition of hydrolysis is the breakdown of a polymer into smaller units by addition of water (Brock and Madigan, 1991). In wastewater treatment applications the processes of hydrolysis summarizes all mechanisms that make slowly biodegradable substrate available for bacterial growth (Gujer *et al.*, 1999). With this broader definition of hydrolysis in wastewater treatment modeling also processes such as chemical dissolution and mass transport processes have to be considered when evaluating hydrolysis rates.

Particles in wastewater

Particle size and particle composition determine rate and mechanism of hydrolysis and degradation in a wastewater treatment system. Strictly speaking, the definition of slowly biodegradable organic matter (X_S) as used in the activated sludge models (Henze, 2000) is only indirectly related to particle size. However, the majority of slowly biodegradable organic matter can be assumed to be in the range from 10^3 amu to 100 µm. Cells can directly take up particles that are smaller than 10^3 amu (Ferenci, 1980; White, 2000). Particles larger than 100 µm are to a large extent removed in the primary sedimentation. In addition to influencing biodegradation, particle size and particle surface properties are also key factors to determine mass transfer and attachment in activated sludge and biofilm systems.

Particle size distributions can be measured either directly in the wastewater sample or indirectly by quantifying after separating particles in different sizes fractions. Direct methods for measuring particle size distributions are photon correlation spectroscopy, laser diffraction, laser light blockage, microscopy followed by image analysis, aperture impedance with orifice detection (e.g. Coulter counter). Only the last method measures both size distribution and particle concentration while all other methods measure only size distributions. No instrument is suitable for characterizing all relevant particles. Instruments have limitations in the size range and in the concentration range that can be measured reliably. Another major limitation of particle sizing instruments is that these instruments do not measure organic matter but number or volume distributions.

Indirect measurement of particle size distribution refers to quantification of particles after separating these particles into different size fractions. Measuring the particle size distribution indirectly has the advantage that chemical or biological properties of particles can be associated with different particle sizes. Methods for particle separation include

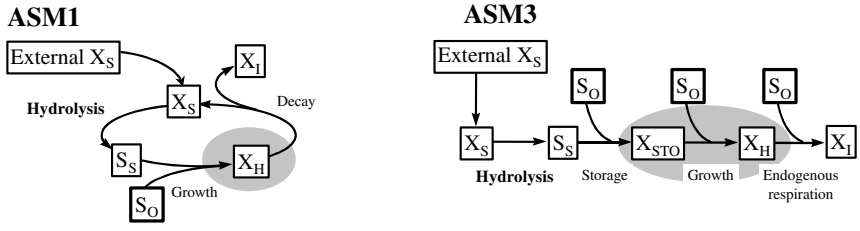


Figure 1 In ASM1, the process of hydrolysis combines primary and secondary hydrolysis (Henze *et al.*, 1987). In ASM3, the degradation of external substrate and the degradation of cellular products is decoupled and the process of hydrolysis refers to primary hydrolysis only (Gujer *et al.*, 1999). (The shaded area marks the components that are considered part of the bacterial cell. S_S = readily biodegradable organic substrates, S_{O2} = dissolved oxygen, X_S = slowly biodegradable substrates, X_H = heterotrophic organisms, X_{STO} = a cell internal storage product of heterotrophic organisms, X_I = inert particulate organic material.)

sedimentation, centrifugation, sieving, microfiltration, ultrafiltration, field flow fractionation, SPLITT (Contado *et al*., 1997), gel filtration chromatography (Levine *et al*., 1991a). Parameters to quantify separated particles include chemical oxygen demand (COD), total organic carbon (TOC), suspended solids, and volatile suspended solids. COD and TOC have the advantage that they can be used to characterize both the soluble and the particulate fractions. In addition to quantifying bulk parameters, it can be useful to differentiate between types of organic matter such as polysaccharides, proteins, fats (Raunkjaer *et al*., 1994). However, even though physical separation methods to characterize wastewater components would be desirable for wastewater characterization, those methods have not been reliable (Wentzel *et al*., 1999).

Vollertsen *et al*., 2001 evaluated different methods to quantify the active biomass in wastewater. They compared respirometric methods with microbiological methods of staining and counting of cells for the determination of total cell biomass (acridine orange and DAPI), physiological state of cells (LIVE:DEAD BacLight) and activity of cells (reduction of the redox dye CTC and microautoradiography). They showed a good correlation between both the respirometric and the staining methods. However, in experiments with the addition of acetate the respirometric method suggested an exponential increase of active biomass while the staining methods did not show such an increase. They concluded that only a small fraction of the cell biomass was responsible for the main part of the substrate uptake.

Overviews of particle size distributions and chemical composition are presented in Table 1 and Table 2, respectively. It should be noted that there are only very few comprehensive studies of characterization of particles. Many studies focus on one particular particle sizing method (limiting the results to either large *or* small particles) or to one particular characterization method (either size distribution of TOC *or* detailed chemical analysis *or* parameter estimation based on respirometry).

Mechanisms for hydrolysis

Hydrolysis of particulate organic matter is likely to be as diverse as the particles and organisms that are involved in the process. Particle sizes range over orders of magnitude, particles are composed of different types of organic matter, and a mixture of different bacteria and also higher organisms are involved in the overall process.

Table 1 Size distribution and chemical composition of organic matter in municipal wastewater (adopted from Levine *et al*., 1991b)

	Percent organic matter contained in size range, µm				
Sample	**< 0.001**	**0.001–1**	**1–100**	**> 100**	**Reference**
Raw influent wastewater	12	15	30	43	Munch *et al*., 1980
Primary effluent	9	48	15	28	Munch *et al*., 1980
Primary effluent	51a	8b	34c	7d	Levine *et al*., 1985
Secondary effluent, activated sludge	28a	3b	20c	49d	Levine *et al*., 1985

a < 0.1 µm; b 0.1–1 µm; c 1–12 µm; d > 12 µm

Table 2 Size distribution and chemical composition of organic matter in municipal wastewater

	Percent COD		
Protein (%)	28	8	12
Carbohydrate (%)	18	12	6
Lipid (%)	31	10	19
Others (%)	22	70	63
Reference	Raunkjaer *et al*., 1994	Henze, 1982	Tanaka *et al*., 1991

Enzymatic hydrolysis

The prokaryotic cell wall disables most bacteria from uptake and degradation of particle and aqueous polymers by phagocytosis. Thus, degradation depends on the extracellular depolymerisation followed by cellular uptake and subsequent metabolisation (Chróst, 1991). Some eukaryotes, like fungi and yeast, also excrete depolymerisation enzymes. Depolymerisation is generally carried out by extracellular hydrolases and/or lyases. Hydrolytic cleavage is characterised by the addition of a water molecule (hydroxyl and proton), while lyase forms an unsaturated and protonated end. Traditionally, degradation of slowly biodegradable matter in wastewater systems has been designated to the process of hydrolysis. That might be due to the fact that most depolymerisation reactions are catalysed by the process of hydrolysis, and only a few through lyase reactions.

Two mechanisms of depolymerisation can be differentiated, and these mechanisms are mediated by different types of enzymes: exo- and endo-enzymes. Exo-enzymes act on a specific bond upstream of the end (normally the non-reducing end), while endo-enzymes act randomly on internal polymer bonds away from the terminal monomers. Thus, degradation of polymers normally involves a range of endo/exo couple actions. Figure 2 shows the most important reactions involved in the degradation of the polysaccharide Dextran.

According to the Enzyme Handbook (Schomburg *et al.*, 1997) 197 extracellular enzymes have been identified. About 145 are hydrolytic, while 11 are lyases. The enzyme substrate specific activity is thought to follow traditional Michaelis-Menten kinetics. However, early studies report first order kinetics (Tauber, 1948) but it is unclear whether that might be due to relatively low substrate affinity and high V_{max} rates. Depolymerisation enzymes only take one substrate and most do not require cofactors or prosthetic groups. They generally show wide temperature and pH ranges, and many have low specificity which makes them versatile for cells scavenging various substrates, providing microorganisms with an adaptive tool during static conditions. This is demonstrated as many extracellular enzymes are synthesized (or synthetically up-regulated) during late exponential and stationary growth conditions. Most extracellular enzymes reported in enzyme databases (like BRENDA, http://www.brenda.uni-koeln.de/) have been studied in vitro, and it is questionable how representative kinetic and stoichiometric coefficients are for in vivo situations. Thus, modeling will have to rely on simplified process mechanisms, and pseudo parameters. Degradation of wastewater organic particles and polymers by extracellular hydrolysis depends on several additional system factors. The local concentration of enzymes, location of the enzymes and product/intermediate transport mechanisms all influence the rate of reaction.

Priest, 1984, reviewed extracellular enzyme synthesis and regulation. The major synthetic mechanism occurs via cotranslational excretion. While depolymerases, those enzymes acting on larger polymers, are located at or outside the cell wall, oligomerases

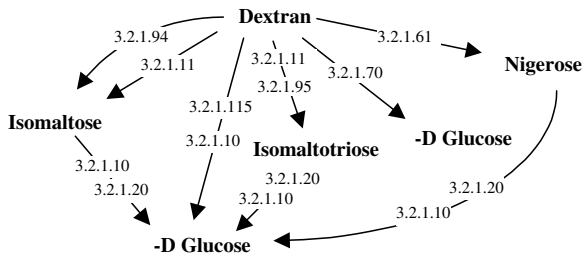


Figure 2 Major routes for enzymatic hydrolysis of Dextran. Each process is indicated by the enzyme involved (EC number), and the end product of the hydrolysis

which act on depolymerase products may be located in the periplasmic space, or even in the cytosol (Juillard *et al*., 1995). While the enzyme activity shows traditional Michaelis-Menten kinetics, the major regulatory mechanism of depolymerising extracellular enzymes is thought to be on a transcriptional level. Some enzymes are constitutive (e.g. amylases, proteases of *Bacillus amyloliquefaciens* and *Bacillus licheniformis)*, but the majority are inducible and subjected to strong catabolite repression. While carbohydrate synthesis is strongly repressed by carbon catabolites, proteases seem to be controlled by nitrogen derivatives. This indicates that the local concentration of depolymerising enzymes depends on the growth condition of the cell. Catabolite repressing transcriptional control mechanisms could be important for understanding hydrolysis mechanisms in wastewater treatment systems as the availability of readily biodegradable matter for growth could result in decreased hydrolysis rates.

Direct degradation of particles by protozoa

Protozoa can directly take up particles in the micrometre range and degrade them intracellulary through phagocytosis (Alberts *et al*., 1994). Thus, for the majority of particles in wastewater no prior extracellular hydrolysis step is required before protozoa can metabolize the particles. To the knowledge of the authors the significance of protozoa on the overall conversion of particulate organic matter in a wastewater treatment system are not well determined. It is feasible, that a large fraction of the organic particles entering a wastewater treatment plant is captured by protozoa. Most protozoa are aerobic and their activity would be significantly reduced during anoxic and anaerobic conditions. Thus, protozoan activity could explain the measured effect of electron acceptor on overall hydrolysis in activated sludge systems (Henze and Mladenovski, 1991). It could be possible, that particles captured by protozoa are not completely metabolized and protozoa may release organic material that can be utilized by bacteria.

Mathematical descriptions of hydrolysis

Municipal wastewater is composed of a complex mixture of organic substrates and biodegradation of different organic fractions has to be described separately to allow for an adequate model under dynamic conditions. Ekama and Marais, 1979, divided the wastewater into two biodegradable fractions that are degraded at two different rates: A readily biodegradable fraction consists mainly of soluble organic matter and a slowly biodegradable fraction that consists of large molecules, colloids and particles that have to be hydrolyzed before degradation. The distinction between these two fractions was made on biological response, not on physical separation (Ekama *et al*., 1986). In the following, stoichiometry and kinetics of hydrolysis are discussed.

Stoichiometry

An overview over different hydrolysis stoichiometries is presented in Table 3. Early models did not consider the process of hydrolysis as a separate process but assumed that there is one bacterial population that consumes both slowly and readily biodegradable (Table 3, Model Nr. 1). Ekama and Marais (1979) suggested that slowly biodegradable organic matter is hydrolyzed and that there are two bacterial populations that grow separately, either on readily or slowly biodegradable organic matter, respectively (Table 3, Model Nr. 2). Results from Frigon *et al*., 2001 measuring rRNA levels in bacteria support this hypothesis of two bacterial populations degrading either predominantly slowly or readily biodegradable substrate. In ASM1 (Henze *et al*., 1987), this idea of two separate populations has been abandoned. A one step hydrolysis process (Table 3, Model Nr. 3) where slowly biodegradable organic substrate is converted into readily biodegradable organic

substrate has been the basis for ASM1, ASM2, and ASM3 (Henze *et al*., 1987; Henze *et al*., 1999; Gujer *et al*., 1999).

More complicated hydrolysis stoichiometries have been suggested for three reasons: (1) Model predictions did not match experimental results and increasing the number of slowly biodegradable fractions increased the degree of freedom in the model and allowed for an improved prediction of measured data (Sollfrank and Gujer, 1991; Orhon *et al*., 1998). (2) An accumulation of hydrolysis products was measured during the experiment, where accumulating products were still too large to be directly metabolized by bacteria (Confer and Logan, 1997a). (3) In biofilm reactors, particle size has to be considered in addition to hydrolysis kinetics because mass transport of slowly biodegradable organic matter within the biofilm strongly depends on size (Janning, 1998). Two approaches can be used to model stoichiometry of multiple slowly biodegradable fractions: Parallel hydrolysis (Table 3, Model Nr. 4) or sequential hydrolysis (Table 3, Model Nr. 5). Parallel hydrolysis allows us to model the utilization of different fractions independent of each other and as a result allows greater flexibility in model calibration. Sequential hydrolysis has to be considered when the accumulation of intermediate hydrolysis products should be described by the model.

Our current understanding of hydrolysis is too limited to judge which of these model stoichiometries is more correct. As discussed above, even in the relatively simple hydrolysis of dextran to glucose there are multiple pathways with multiple enzymes, intermediates and rates and hydrolysis is a mixture of sequential and parallel processes (Figure 2). In addition to bacterial hydrolysis, protozoa are competing for particulate organic

Table 3 Hydrolysis and growth stoichiometries

Processes	$X_{S,1}$	$X_{S,2}$	$X_{S,3}$	$X_{S,ads}$	S_S	S_{O2}	$X_{H,1}$	$X_{H,2}$
Model Nr. 1: Direct growth on both soluble and particulate organic matter								
Growth on $X_{S,1}$	$-1/Y_H$					$-(1-Y_H)/Y_H$	1	
Growth on S_S					$-1/Y_H$	$-(1-Y_H)/Y_H$	1	
Model Nr. 2: Two biomass system								
Adsorption of hydrolysable COD ($X_{S,1}$)	–1			1				
Direct growth on adsorbed COD				$-1/Y_H$		$-(1-Y_H)/Y_H$	1	
Growth on soluble COD					$-1/Y_H$	$-(1-Y_H)/Y_H$		1
Model Nr. 3: One step hydrolysis								
Hydrolysis of hydrolysable COD ($X_{S,1}$)	–1				1			
Growth					$-1/Y_H$	$-(1-Y_H)/Y_H$	1	
Model Nr. 4: Parallel hydrolysis								
Hydrolysis of slowly hydrolysable COD ($X_{S,1}$)	–1				1			
Hydrolysis of intermediate hydrolysable COD ($X_{S,2}$)		–1			1			
Hydrolysis of rapidly hydrolysable COD ($X_{S,3}$)			–1		1			
Growth					$-1/Y_H$	$-(1-Y_H)/Y_H$	1	
Model Nr. 5: Sequential hydrolysis								
Hydrolysis of slowly hydrolysable COD ($X_{S,1}$)	–1	1						
Hydrolysis of intermediate hydrolysable COD ($X_{S,2}$)		–1	1					
Hydrolysis of rapidly hydrolysable COD ($X_{S,3}$)			–1		1			
Growth					$-1/Y_H$	$-(1-Y_H)/Y_H$	1	

Where

Model No. 1: Direct growth on both soluble and particulate organic matter. Hydrolysis is not considered as a separate process (e.g. Stenstrom, 1975).

Model No. 2: Two biomass system (Ekama and Marais, 1979, Dold *et al*., 1980, Frigon *et al*., 2001).

Model No. 3: One step hydrolysis (e.g. Henze *et al*., 1987 (=ASM1); Henze *et al*., 1995 (=ASM2), Henze *et al*., 1999 (=ASM2d), Orhon *et al*., 1999 (=ASM3), Sollfrank, 1988; Larsen, 1992; Spanjers and Vanrolleghem, 1995).

Model No. 4: Parallel hydrolysis (e.g. Sollfrank and Gujer, 1991; Janning, 1998; Orhon *et al*., 1998, Vollertsen and Hvitved-Jacobsen, 1999, Vollertsen, 1998).

Model No. 5: Sequential hydrolysis (e.g. Bjerre, 1996; Confer and Logan, 1997a; Spérandio and Paul, 2000).

$X_{S,1}$, $X_{S,2}$, $X_{S,3}$ = slowly biodegradable organic matter (in models with multiple X_S fraction $X_{S,1}$ is slowly and $X_{S,3}$ is rapidly hydrolysable. $X_{S,ads}$ = adsorbed X_S, S_S, readily biodegradable organic matter, S_{O2} = oxygen, $X_{H,1}$, $X_{H,2}$ = separate heterotrophic bacterial populations

substrate (Ratsak *et al.*, 1996). In the end, selection of a model structure should be guided by the necessary detail for the specific simulation and available data for model calibration.

Kinetics

The overall process of hydrolysis is described by combining model stoichiometry and model kinetics. In the following section, the kinetic expressions associated with model stoichiometry are discussed. The form of the kinetic expressions turned out to be largely independent of the model stoichiometry. Dold *et al.* (1980) developed a kinetic expression for hydrolysis based on Stenstrom (1975) that assumed that slowly biodegradable organic matter adsorbs to the surface of the organisms and is degraded by extracellular enzymes. This results in surface limited hydrolysis kinetics as described by Model Nr. V and VI in Table 4. Based on experimental results with variations in sludge age and sludge concentration, Dold *et al.* (1980) suggested that hydrolysis kinetics cannot be sufficiently described with simple Monod kinetics (Table 4, Model IV). A subject of ongoing debate has been, whether hydrolysis rates are influenced by redox conditions as can be seen by changes in the activated sludge models. In ASM1 and ASM2 (Table 4, Model VI) a reduction factor η reduced hydrolysis rates if no oxygen was present. In ASM1 η was 0.4 and in ASM2 η was 0.1 or 06 for anaerobic and anoxic conditions respectively (values for 20°C). In ASM3, no reduction factor was considered any more. This modification was supported by Goel *et al.* (1998b) who showed that enzyme activity was not significantly affected by redox conditions.

Simplified models for hydrolysis kinetics have been proposed as zero order, first order, or saturation type kinetics (Table 4, Model 0-IV). Most of these models have been developed for specific situations (very high or very low substrate to microorganism ratios) and it can be shown that under these conditions the surface limited hydrolysis rate expression in Model V can be simplified: For $X_{S,i} << X_H$ the original Model V can be approximated with first order substrate kinetics (Model I, with $k_{H,I} = k_{H,IV} / K_X$). For $X_{S,i} >> X_H$ Model V can be approximated also with a first order model – this time in terms of the biomass concentration (Model II, with $k_{H,II} = k_{H,IV}$). Likewise Model IV simplifies to Model III and II for $X_{S,i} << X_H$ and $X_{S,i} >> X_H$, respectively.

Table 4 Reaction rate expressions

Nr.	Kinetic expression	References
0	$k_{H,0}$	Larsen, 1992, Cliff, 1980, Andrews and Tien, 1977, Dennis and Irvine, 1981, Tsuno, 1978
I	$k_{H,I} \cdot X_S$	Gujer, 1980, Henze and Mladenovski, 1991, Sollfrank and Gujer, 1991, Janning, 1998, Kappeler and Gujer, 1992, San Pedro *et al.*, 1994, Spérandio and Paul, 2000, Spanjers and Vanrolleghem, 1995, Goronszy and Eckenfelder, 1991
II	$k_{H,II} \cdot X_H$	Goel *et al.*, 1997
III	$k_{H,III} \cdot X_S \cdot X_H$	Eliosov and Argaman, 1995, Argaman, 1995, Sollfrank, 1988, Mino *et al.*, 1995
IV	$k_{H,IV} \cdot \frac{X_S}{K_{X,IV} + X_S} X_H$	Larsen, 1992, Mino *et al.*, 1995, Goel *et al.*, 1998a
V	$k_{H,V} \cdot \frac{X_S / X_H}{K_{X,V} + X_S / X_H} X_H$	Stenstrom, 1975, Mino *et al.*, 1995, Janning, 1998, Gujer *et al.*, 1999 (=ASM3)
VI	$k_{H,VI} \cdot \left(\frac{X_S / X_H}{K_{X,VI} + X_S / X_H} \cdot \frac{S_{O2}}{K_{O2} + S_{O2}} X_H + \eta_H \cdot \frac{X_S / X_H}{K_{X,VI} + X_S / X_H} \cdot \frac{K_{O2}}{K_{O2} + S_{O2}} X_H \right)$	Dold *et al.*, 1980, Henze *et al.*, 1987 (=ASM1), Henze *et al.*, 1995 (=ASM2), Henze *et al.*, 1999 (=ASM2d)

Experiments on hydrolysis

Experiments have been designed to evaluate mechanisms and location of hydrolysis and to quantify the influence of hydrolysis on substrate utilization. Four experimental approaches can be differentiated according to measured parameters: (1) Measurement of specific hydrolytic enzymes; (2) Measurement of specific hydrolytic intermediates or specific end products; (3) Overall mass balances for bulk organic parameters, (4) Measurement of respiration rates to quantify bacterial activity. Applications of these experimental approaches with specific advantages and shortcomings are discussed below.

Experimental studies of enzyme activity in wastewater treatment

Assuming that enzymatic breakdown is the dominant mechanism in hydrolysis, then the quantification of enzymatic activity is the most direct way to study mechanisms and location of hydrolysis. Because of the difficulties in measuring the amount of an enzyme in the conventional units of mass or molar concentration, the accepted unit of enzyme activity is defined in terms of reaction rate (Holme and Peck, 1993). Degradation of a defined substrate for a specific enzyme is measured over time and enzyme activity is expressed in enzyme units, where one unit will result in the conversion of 1 μmol of substrate to product in 1 min under specified conditions. The direct determination of enzymes has limited applicability when applied to mixtures of substrate such as wastewater. Both the composition of the organic substrate and the corresponding enzymes are generally not known.

Numerous studies of enzymatic activity of enzymes in activated sludge have been used to characterize the activity of activated sludge (Vaicum *et al*., 1965; Klapwijk *et al*., 1974; Teuber and Brodisch, 1976; Dold *et al*., 1991). Several studies focused on the activity of extracellular hydrolases (Thiel and Hattingh, 1967; Sridhar and Pallai, 1973; Nybroe *et al*., 1992; Boczar *et al*., 1992; Frolund *et al*., 1995). Richards *et al*., 1984 screened a number of activated sludge plants for the activity of eight enzymes, among these α-Glucosidase and Protease (both extracellular hydrolytic enzymes). They found indications of positive correlation between hydrolytic activity and COD loading rate. Nybroe *et al*. (1992) correlated activated sludge enzymatic activity with biomass concentration and microbial activity. However, α-Glucosidase did not seem to be affected by activity, indicating constitutive synthetic regulation. San Pedro *et al*. (1994) did not find any significant effect of biomass concentration on starch hydrolysis rate. However, they did see an effect of acclimation time, which may indicate an inducible long-term effect. Hydrolysis of starch correlated well with first order hydrolysis kinetics, and interestingly enough the activity of hydrolysis did not seem to be affected by changing electron acceptor conditions. This appears to be contrary to Goel *et al*. (1997) who identified a strong dependency between incubation electron acceptor conditions and hydrolytic activity in batch operated *Bacillus amyloliquefaciens* and *Pseudomonas saccharophilia* pure cultures. However, Goel *et al*. found that it was not the specific activity of hydrolytic enzymes that were affected, but rather the hydrolytic enzyme synthesis rate. The inactivation rate for hydrolytic enzymes was shown to be in the order of hours or days (Goel *et al*., 1998b). Assuming that retention times under anaerobic or anoxic conditions are low relative to the inactivation rates of enzymes could explain why enzymatic hydrolysis rates were not affected under changing redox conditions in wastewater treatment plants but that in long term batch experiments redox conditions significantly influence hydrolysis rates (Henze and Mladenovski, 1991). In a SBR activated sludge system treating a synthetic wastewater Goel *et al*. (1999) demonstrated that there is no electron acceptor effect on the specific activity of α-Glucosidase, Protease and Alkaline- and Acidic phosphatase.

Location of extracellular hydrolytic activity was studied by Goel *et al*. (Goel *et al*., 1997; Goel *et al*., 1998a; Goel *et al*., 1999). Their major conclusion was that bulk phase

extracellular hydrolysis was significant in two pure cultures studied, while the activity in SBR sludge was associated with the sludge flocs. Similar results were obtained by Boczar *et al*. (1992) and Dold *et al*. (1991) who showed that activated sludge contained most hydrolytic activity, with negligible bulk water activity. Frolund *et al*. (1995) later found that Esterase and Leucine aminopeptidase activity was 18–32 times higher in the activated sludge flocs compared to bacterial culture. This strongly suggests that the extracellular matrix may immobilize large amounts of extracellular enzymes. Confer and Logan (1998) found that leucine aminopeptidase and α-glucosidase activity was cell mass associated, both for biofilm and suspended growth batch systems. In trickling filter effluent, hydrolysis was also predominantly cell-associated. Guellil *et al*. (2001) extracted EPS from activated sludge and compared enzymatic activity in the bulk water and in the EPS extract. They found that protein hydrolysis mainly resulted from the enzymatic activity of EPS, whereas glycolytic activity was mainly present in the organic colloidal fraction of the wastewater. Vetter *et al*. (1998) concluded that released extracellular enzymes provide individual bacteria with a powerful feeding mechanism, especially in typical wastewater conditions (high surface area, high particulate organic concentrations). By comparing net energy gain, they showed that up to a limited distance, cell free enzymes provide an important carbon source even without taking mutualistic effects into account. If enzymes released to the bulk phase play a significant role in hydrolysis then the hydraulic retention would become important as very short hydraulic retention times (e.g. in high rate biofilm reactors) would result in a washout of bulk phase enzymes (Larsen and Harremoës, 1994).

Measurement of substrates and hydrolytic intermediates

Experiments have been quantifying the removal of a defined substrate or quantifying the production of a specific intermediate or end product. This procedure requires a substrate with well defined hydrolysis products. Thus, experiments have mainly been carried out using artificial substrates.

Haldane and Logan, 1994 monitored the production of intermediates during the hydrolysis of dextran (70,000 average molecular weight) in axenic culture using a dextran-degrading bacterium isolated from wastewater. Size fractions of carbohydrates were extracted by ultrafiltration with nominal cut offs at 1,000 and 10,000 amu. It was shown that significant amounts of carbohydrates accumulated both in the small (<1,000 amu) and the intermediate (>1,000 and <10,000 amu) size fractions. It can be questioned whether similar accumulation of intermediates will also occur for more complex substrates and mixed cultures. Confer and Logan (1997a,b) evaluated the degradation of proteins and carbohydrates (bovine serum albumin, dextran, and dextrin) in a biofilm system and showed a similar release of intermediates in the bulk phase. A conceptual model was developed where large molecular size molecules diffuse into the biofilm where hydrolysis occurs and hydrolysis products diffuse back into the bulk phase even though these hydrolysis products are in the size range that can be directly taken up by bacteria. Ubukata (1999) used dextrin as a model substrate and monitored the disappearance of macromolecules and individual amino acids during a batch experiment. In these experiments, no hydrolysis products (e.g. glucose, maltose, maltotriose) were detectable in the bulk phase. Larsen and Harremoës, 1994, studied hydrolysis of starch in a biofilm reactor. They suggested the process of hydrolysis to take place in the bulk phase caused by extracellular enzymes that are released to the bulk phase. The location of the enzymatic depolymerisation is especially important for the understanding of hydrolysis of particulate matter in biofilm systems as hydraulic retention times in biofilm systems can be an order of magnitude smaller compared to activated sludge systems and hydrolytic intermediates (or enzymes) released to the bulk phase could be washed out of the system. In seawater, where bacteria attaching to organic

particles are responsible for hydrolysis, it was shown that bacteria hydrolyze more organic substrate than they can use for their own metabolism resulting in a significant release of hydrolysis products to the bulk phase (Kepkay, 1994). Overall, the release of intermediates into the bulk phase appears to be system related depending on the substrate to biomass ratio and on the complexity of substrate and microbial population.

Mino *et al.*, 1995, and San Pedro *et al.*, 1994, monitored the fate of starch in batch experiments. The following intermediates were measured: (1) bulk starch, (2) bulk low molecular glucose, (3) exocellular starch, (4) exo- and intracellular low molecular glucose, (5) intracellular glycogen. During the experiments bulk and exocellular starch rapidly disappeared suggesting a rapid hydrolysis of starch to low molecular short chain saccharide or glucose. It was shown that electron acceptor conditions had no influence on those measured hydrolysis rates. For the S_0/X_0 conditions in the experiment the biomass concentration had no significant influence on hydrolysis rate of starch. Activated sludge for these experiments was derived from a SBR that had been inoculated with sludge from a municipal wastewater treatment plant. Acclimation occurred in this SBR where measured hydrolysis rates for starch increased by a factor of two to three over a period of 70 days showing that bacteria in activated sludge have to acclimatize to the hydrolysis of starch.

Henze and Mladenovski, 1991, studied the hydrolysis of raw municipal wastewater and they monitored the increase of bulk phase ammonia as a specific hydrolysis product. They assumed that ammonia was not consumed during the course of their experiments (4 days) as biomass growth was negligible. Different from Goel *et al.* (1998b) they found a significant reduction of hydrolysis rates under anoxic or anaerobic conditions.

Measurement of bulk parameters

Mass balances in terms of bulk parameters for organic components can be used to evaluate the overall extent and rate of hydrolysis. Using bulk parameters allows us to quantify hydrolysis in complex systems, using for example wastewater as a slowly biodegradable substrate. Janning *et al.*, 1998, prepared a complete carbon balance based on organic (TOC) and inorganic (TIC) carbon in the in- and effluent of a pilot scale biofilm reactor operated with pre-screened wastewater. Based on these mass balances they could show that only 15% of the accumulated TOC was transformed during their 24 hour experiments. Eliosov and Argaman (1995) quantified hydrolysis of particles extracted from wastewater by monitoring VSS and quantifying a biomass VSS through respiration rate measurements after the addition of glucose. In their experiments, they found a decreasing hydrolysis rate with increasing particle size.

Measurement of bacterial respiration

Dynamic experiments measuring respiration rates were the basis for introducing two biodegradable organic fractions (Ekama and Marais, 1979; Dold *et al.*, 1980). Increasing the number of wastewater fractions allowed us to improve the description of measured results. However, the model parameters (hydrolysis stoichiometry, kinetics, and the amount of slowly biodegradable COD) cannot be estimated based on the respiration rate experiments (Spanjers and Vanrolleghem, 1995). To determine the amount of slowly biodegradable COD, ASM1 (Henze *et al.*, 1987) suggested to measure or estimate all other COD fractions and then determine the slowly biodegradable COD based on a mass balance. Kappeler and Gujer, 1992, have suggested curve fitting of the amount of slowly biodegradable substrate and the hydrolysis rate constant (note that Kappeler and Gujer assumed a first order hydrolysis rate (Table 4, Nr. I) that is comparable to hydrolysis kinetics in ASM1 only for low concentrations of slowly biodegradable substrate). Spanjers and Vanrolleghem, 1995, also estimated slowly biodegradable organic matter from respiration

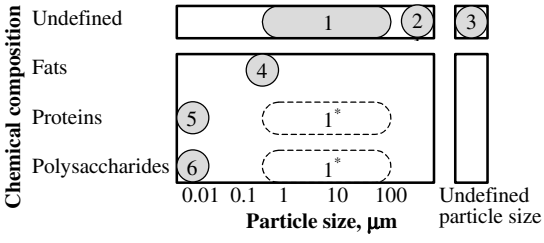
rate measurements. However, they divided the slowly biodegradable organic matter into two fractions and curve fitting allowed only estimation of the rapidly hydrolysable organic fraction.

Vollertsen and Hvitved-Jacobsen (1999) divided the slowly biodegradable organic matter into three fractions that are hydrolyzed in parallel (Table 3, Model Nr. 4). Subsequently, they used batch experiments with respiration rate measurements to quantify all biodegradable organic matter fractions and hydrolysis rates for the three parallel hydrolysis processes. Spérandio and Paul, 2000 used a combination of two batch experiments with either high or low initial substrate to biomass ratios. They described hydrolysis as a sequential two step process (adsorption followed by hydrolysis) and estimated wastewater composition and the hydrolysis rate based on simultaneous parameter estimation from these two batch experiments.

The basic assumption of experiments using respiration rate measurements to quantify hydrolysis is that hydrolysis is the rate limiting step that determines respiration rates (Dold *et al.*, 1980; Arvin and Harremoës, 1990). For starch, Goel *et al.*, 1998c, showed that even though hydrolysis of starch was limiting the overall reaction rate, the accumulation of glycogen as a storage product was observed. Thus, it was concluded that the respiration rate cannot directly be linked to substrate hydrolysis. Overall, respirometry will be a valuable tool to estimate wastewater composition and reaction rates (Vanrolleghem *et al.*, 1999). However, there is a large uncertainty associated with hydrolysis parameters extracted from parameter estimation.

Hydrolysis in wastewater treatment applications

Hydrolysis mechanisms, rates and locations depend on type and concentration of the slowly biodegradable substrate and on the microbial population (Goel *et al.*, 1998a). Caution should be employed when applying results from experiments with a simple substrate or a pure culture to wastewater treatment applications. Experimental investigations often use defined substrates that allow us to trace intermediates and enzyme activity. Many studies have used starch as a model compound that can easily be quantified and the enzymes involved in hydrolysis are known. In Figure 3, an overview of substrates used in hydrolysis studies is shown in terms of chemical composition and particle size. Many studies measured hydrolysis rates using either raw wastewater or large particles extracted from raw



Legend:

1 (Filtered wastewater): Guellil *et al.*, 2001 (1*): Note that proteins and polysaccharides were measured

2 (Filtered, settled or centrifuged wastewater or sewer solids): Eliosov and Argaman, 1995, Janning *et al.*, 1998, Vollertsen and Hvitved-Jacobsen, 1999

3 (Raw wastewater): Henze and Mladenovski, 1991

4 (Fats): Sprouse and Rittmann, 1990

5 (Bovine Serum Albumin): Ubukata, 1992, Confer and Logan, 1997a

6 (Starch, dextrin): Ubukata, 1992, Larsen and Harremoës, 1994, Haldane and Logan, 1994, San Pedro *et al.*, 1994, Goel *et al.*, 1997, Goel *et al.*, 1998a, Goel *et al.*, 1998c, Confer and Logan, 1997b

Figure 3 Particle size and particle composition used for hydrolysis experiments

wastewater. Bovine serum albumin and starch have been frequently used as defined substrates representing protein or polysaccharide, respectively. It is unclear how far particle size is affecting hydrolysis rates as limited research has been done using defined synthetic or particles extracted from wastewater in the range from 0.1 to 100 μm. However, according to Levine *et al*. (1991b) this size fraction contains the majority of particulate organic matter in primary effluent (Table 1).

Difference between activated sludge and biofilm processes

Most studies on hydrolysis have been done using suspended cultures rather than biofilms. However, recent research shows that the overall effect of hydrolysis is different in biofilms compared to activated sludge systems (Janning *et al*., 1998). In biofilm systems, the fraction of particulate organic matter that is not utilized by bacteria appears to be significantly larger compared to activated sludge systems. However, most mathematical models for biofilms are based on hydrolysis stoichiometries and kinetics originally developed for activated sludge systems.

Two mechanisms can lead to a decreased overall conversion of particulate organic matter in biofilm reactor systems: Hydrolysis requires direct contact between particulate organic matter and bacteria. It has been demonstrated that small particles (1 μm) can readily penetrate a biofilm matrix (Drury *et al*., 1993). However, mass transport into the biofilm decreases with increasing particle size. This situation is different from activated sludge flocs, which have a more open structure and a larger relative surface area. A second mechanism is related to the retention time of particulate organic matter in biofilm reactors. In activated sludge systems particulate organic substrate is enmeshed in the sludge floc and effectively has the same retention time in the system as the bacteria. In biofilm systems, it can be assumed that large particles accumulate at the biofilm surface where preferential removal during backwashing occurs. Thus, the retention time of particulate organic substrate in biofilm reactors can be significantly smaller compared to the retention of bacteria. In Figure 4, characteristic times of reactions are compared to retention times in wastewater

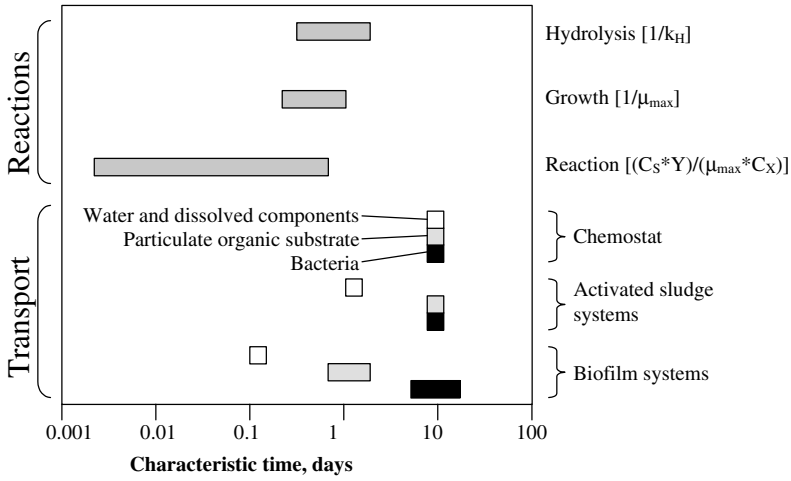


Figure 4 Characteristic times for reactions and transport in suspended cultures and biofilm systems. (Characteristic times of transport processes equal hydraulic and solid retention times for dissolved and particulate components, respectively. For biofilm systems, it is assumed that the particulate organic substrate attaches to the surface of the biofilm where preferential detachment may occur resulting in a characteristic time that is smaller compared to the biomass retention time in the same system (Morgenroth and Wilderer, 2000). Characteristic times for simplified reaction processes are shown in [.] assuming μ_{max} = 1 – 5 d^{-1}, k_H = 0.5 – 3 d^{-1}, C_X = 4 g/m^3, C_S = 0.1 – 5 g/m^3, Y = 0.5

treatment systems. If the characteristic time of a process is significantly smaller than the retention time of the reactant then in can be expected that most of the reactant will be removed in the process. In Figure 4 it can be seen that the retention time of particulate organic substrate in a biofilm reactor is of the same order of magnitude as the characteristic times of hydrolysis. This means that slowly hydrolysable substrate may be removed from the biofilm reactor through backwashing before hydrolysis can occur. These conclusions are in accordance with the observations of Janning *et al*. (1998) who showed that in a biofilm reactor with backwashing a large fraction of particulate organic matter is removed through backwashing and is not oxidized.

Conclusions

- Mechanisms of hydrolysis in wastewater treatment applications (mixed culture, mixed substrate) are not well understood.
- In mathematical models, the process of hydrolysis must be adequately described to be able to predict spatial and temporal availability of organic substrate for nutrient removal processes (denitrification and biological phosphorus removal).
- Currently available models of hydrolysis work well for typical applications in wastewater treatment. However, for new treatment processes, or if the composition of the wastewater is atypical, current modeling approaches may not be applicable any more.
- Research on hydrolysis is based on four experimental approaches quantifying (1) Enzymes, (2) hydrolytic intermediates, (3) bulk parameters, or (4) bacterial respiration rates. The first two approaches allow us to study specific mechanisms involved in hydrolysis but are often restricted to specific substrates (e.g. starch). The latter two approaches allow us to evaluate the overall processes but may not allow us to study specific mechanisms involved in the hydrolysis process.
- Future research should evaluate mechanisms of hydrolysis processes using mixed substrates and mixed cultures. Differences between hydrolysis in traditional activated sludge systems and other treatment technologies (membrane activated sludge system, granular sludge system, biofilm reactors) should be evaluated.

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