Hydrolytic enzymes in sewage sludge treatment: A mini-review

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Abstract

Biological wastewater treatment processes can be classified as either aerobic or anaerobic. These two biological treatment processes are each characterised by groups of micro-organisms and their associated enzymes. Hydrolytic enzymes secreted by these micro-organisms are vital for the rate-limiting step of hydrolysis in the treatment of highly polymeric substrates present in sewage sludge. In this mini-review, the effects of mass transfer limitation, metabolic intermediates, extracellular polymeric substances (EPS), electron acceptor conditions and pH and temperature on the activity of these enzymes are summarised. The most salient and current perspectives of the significance and the role that hydrolytic enzymes play in sewage sludge treatment are highlighted.

Keywords: EPS, floc, hydrolases, pH, sewage, sludge, temperature

Introduction

Wastewater treatment processes can be divided into three classes; physical, chemical and biological. Biological treatment processes, in turn, can be classified as either aerobic or anaerobic. Each of these two classes of wastewater treatment has distinct associated advantages and disadvantages (Table 1). From an enzymatic point of view, aerobic and anaerobic processes are each characterised by groups of micro-organisms and their associated enzymes. The goal of this mini-review is to summarise the effects of mass transfer limitation, metabolic intermediates, EPS, electron acceptor conditions and pH and temperature on the activities of hydrolytic enzymes in sewage sludge treatment. The most salient and current perspectives on the significance and the role that hydrolytic enzymes play in sewage sludge treatment are highlighted.

Mass transfer limitations imposed by size restriction due to bacterial membrane dimensions

The removal of organic matter by biological oxidation (e.g. in the activated sludge process) depends on the activity of a mixed population of heterotrophic organisms that is able to utilise either oxygen or nitrate as the terminal electron acceptor in their metabolic reactions (Nybroe et al., 1992). The target pollutants to be destroyed must undergo mass transfer into the bacterial cells in order to take part in metabolic reactions, but only monomeric and oligomeric substrates (< 1 000 Da molecular weight) are able to cross bacterial membranes through cell-specific active transport processes (Cadoret et al., 2002). Bacteria in the activated sludge degrade the complex organic matter (polymeric substrates such as proteins, lipids and carbohydrates) into low molecular-weight intermediates by the action of extracellular hydrolases (Nybroe et al., 1992). These low molecular-weight compounds are in turn assimilated by the cells and used as a source of energy and carbon. Stepwise depolymerisation (e.g. hydrolysis) of highly polymeric substrates is usually the first and overall rate-limiting step for the mineralisation of organic matter in activated sludge and anaerobic digested sludge treatment systems (Frølund et al., 1995; Gessesse et al., 2003; Higuchi et al., 2005; Whiteley et al., 2002a).

These rate-limiting steps are central to several mathematical models that have been developed explaining the biochemistry of biological wastewater treatment processes and predicting plant performance to assist wastewater treatment plant design engineers. Such models are one of the most successful ways of translating biological phenomena into process design parameters. The models all utilise kinetic parameters based upon the initial wastewater breakdown rate – i.e. hydrolysis by exoenzymes. For example, early work on aerobic treatment led to the creation of the widely utilised Activated Sludge Model No. 1 (ASM1, Henze et al., 1987). This was a relatively basic model and has since been revised and expanded to create ASM2 and ASM2d, which include phosphorus removal (Henze et al., 1995, 1999) and later ASM3, in which biological substrate transport into cells and subsequent intracellular storage (i.e. bacterial membrane size restriction limitations) were proposed as the most important mechanism of carbon and nitrogen utilisation and hence removal from wastewater (Gujer et al., 2000). All of the ASMs use biological growth kinetics to describe the activity of the biomass, with increasing model complexity owing to the cumulative addition of model components such as different biochemical processes, each of which has its own kinetic parameters. The kinetic parameters are assumed not to change (i.e. are treated as constants), and are further assumed to depend on the process configuration, substrate type and quantity and mean cell retention time alone.

Another model often used to allow the modelling of anaerobic wastewater treatment processes is anaerobic digestion model no. 1 (ADM1) (Batstone et al., 2002). The problem with this generic model is that it makes use of some oversimplified reactions in solid degradation processes. Yasui et al. (2008) investigated a modified ADM1 structure for modelling municipal primary sludge hydrolysis. Based on the results obtained from this study, modifications in the model structure of ADM1 were proposed to improve the modelling of primary sludge solid degradation in anaerobic digesters. Three biodegradable fractions were classified in this modified model:

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TABLE 1 Advantages and disadvantages of some common wastewater treatment unit processes			
Process	**Advantages**	**Disadvantages**	**Waste products**
Aerobic suspended growth processes, e.g. activated sludge: Widely used in a variety of modifications (contact stabilisation, oxidation ditches, extended aeration, deep shaft etc). Vigorous aeration of flocs produces aerobic biological activity, followed by sedimentation.	Rapid purification possible at optimum settings. Ideal for medium-high strength organic wastes containing natural pollutants, e.g. meat processing, brewing, distillery waste and sugar / starch waste. Small footprint compared with, e.g. aerobic ponding systems.	Aeration and agitation rates critical. Larger land areas normally required than when using, e.g. membrane bioreactors. Sludge quality critical (bulking & rising can occur). Can produce malodours.	Sludge
Aerobic attached growth processes, e.g. biological filtration: High rate, large surface area plastic packings promote growth of aerobic bacteria. Bed is irrigated using fixed or rotary distributor, while natural or forced air rises up through packing. 'Filtration' is a bad name.	Low energy system. Widely used for high rate roughing. Flexible by virtue of packing top-up.	Susceptible to biocides and flies. Recycle may be needed to ensure packing wetted during periods of low feed.	Sludge from downstream clarifier. Odours may need treating especially in summer.
Aerobic attached growth processes, e.g. rotating biological contactors (RBCs): Consist of a shaft onto which are fitted large diameter plastic sheet discs or cylindrical cages containing random or plastic media. The media is partly submerged in the effluent and the assembly is rotated slowly.	Ideal where space is at a premium and good for low and medium strength effluents in large volumes.	Can suffer mechanical damage on start-up due to the imbalanced loads resulting from uneven biofilm growth.	Sludge
Anaerobic suspended / attached growth processes, e.g. high rate anaerobic systems: Can be upflow and downflow filters, fluidised beds, sludge blankets etc. Oxygen excluded to encourage growth of methanogenic bacteria.	Ideal for high strength organic wastes containing natural pollutants, e.g. meat processing, brewing, distillery waste and sugar/starch waste. Produces low amounts of sludge. Possible to use the methane as a fuel.	Strong foul odours may occur if reactors are not isolated from the wider environment. Long retention times needed so large volume reactors required compared with e.g. aerobic systems with 8 h HRT.	Biogas (valuable by-product) and high strength sludge (also has a commercial value)

- An easily hydrolysable substrate with a degradation similar to that of slowly degradable compounds,
- A substrate fraction with a degradation similar to the lysis of a biomass fraction, and
- A substrate requiring disintegration before subsequent hydrolysis, which is representative of large-sized particles in primary sludge (Yasui et al., 2008).

Extracellular enzymes: Ectoenzymes and exoenzymes

Exoenzymes (such as lipases, glucosidases, proteases, etc.) (Table 2) (Frølund et al., 1995; Nybroe et al., 1992) can originate from one of three key sources, namelythe sewage influent; the activated sludge via cell autolysis; or as enzymes that are actively secreted by the cells. Furthermore, exoenzymes are either cell surface bound (ectoenzymes), in free form (exoenzymes) in water or adsorbed within the extracellular polymeric substances (EPS) of the sludge matrix (Cadoret et al., 2002; Frølund et al., 1995; Vavilin et al., 1996). Higuchi et al. (2005) have divided the extracellular enzymes in anaerobic digested sludge into two classes: 'cell-free enzyme' dispersed in the bulk liquid and 'cell-bound enzyme' associated with the microbial cell surface. Using fluorescent *in situ* hybridisation, Higuchi et al. (2005) have indicated that cell-bound alpha-amylase is mainly responsible for the hydrolysis of digested sludge. The degree of contact between the microbial cells and their substrates is thus of crucial importance. Boczar et al. (1992) reported that the amount of exoenzymes dissolved in water is negligible. Frølund et al. (1995) and Goel et al. (1998b) later reported that the hydrolases in sludge were mostly bacterial cell associated or floc associated (embedded in the EPS matrix), but not present in the bulk liquid. Guellil et al. (2001) demonstrated that the proteolytic activity in activated sludge flocs were mainly found in the EPS, while the glycolytic activity was associated with the organic colloidal fraction of the wastewater. Cadoret et al. (2002) then stated that the localisation of extracellular enzymes is not clearly established, and that the distribution of extracellular enzymes between the cell surface and the EPS is still quite unknown. Lastly, Whiteley et al. (2002a) showed that protease and phosphatase enzyme activities were predominantly associated with the organic particulate matter of the primary sewage sludge.

Exoenzyme activity is mostly confined to the hydrolase (Class 3) enzymes, most notably the lipases, phosphatases, glucosidases, and proteases (Boczar et al., 1992; Frølund et al., 1995; Nybroe et al., 1992). Jain et al. (1992) showed that the concentration of these hydrolases, and the contact that exists between these enzymes and their substrates, were very important in their modelling studies of complex particulate substrates during anaerobic digestion. Over a decade later, Novak et al. (2003) observed that the activity of these hydrolases declined during both aerobic and anaerobic digestion. Under aerobic conditions, however, a rapid loss of glucosidase activity over the first ten days was associated with a concomitant accumulation of polysaccharide material (Novak et al., 2003). In fact, a number of authors have shown the benefit of enzyme addition or pre-treatment on the

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TABLE 2 The Class 3 hydrolases of importance to wastewater treatment				
EC number*	**Common name**		**Sludge type**	**Reference**
3.1.1	Lipases	Lipases	Anaerobic-aerobic	Goel et al. (1998a)
		Lipases	Anaerobic	Whiteley et al. (2003a)
		Lipases	Anaerobic	Whiteley et al. (2003b)
		Lipases	Activated	Gessesse et al. (2003)
		Lipases	Anaerobic (dairy)	Leal et al. (2006)
3.1.3	Phosphatases	Acid phosphatase	Anaerobic-aerobic	Goel et al. (1998b)
		Phosphatases	Anaerobic	Whiteley et al. (2002a)
3.2.1	Glucosidases	α-glucosidase	Anaerobic-aerobic	Goel et al. (1998b)
		α-glucosidase	Activated	Nybroe et al. (1992)
		α-glucosidase	Activated	Cadoret et al. (2002)
		α-amylase	Activated	Cadoret et al. (2002)
		α-amylase	Anaerobic digested	Higuchi et al. (2005)
			activated	Guellil et al. (2001)
		β-glucosidases	Anaerobic	Whiteley et al. (2003b)
		β-glucosidases	Anaerobic	Whiteley et al. (2002b)
		Amylases and α-glucosidase	Aerobic, anaerobic and anoxic	Goel et al. (1998a)
3.4	Proteases	Protease	Anaerobic-aerobic	Goel et al. (1998b)
		Protease	Anaerobic-aerobic anoxic	Goel et al. (1998a)
		Protease	Activated	Cadoret et al. (2002)
		Protease	Activated	Guellil et al. (2001)
		Protease	Anaerobic	Whiteley et al. (2002a)
		L-Leu-aminopeptidase	Activated	Cadoret et al. (2002)
		protease		
		alanine-aminopeptidase	Activated	Gessesse et al. (2003)
			Anaerobic	Watson et al. (2004)
			Activated	Nybroe et al. (1992)

**Enzyme Commission Number*

conditioning of wastewater solids (primary sewage sludges) and enhancement of the degree of dewaterability of anaerobically digested biosolids (Ayol, 2005; Ayol and Dentel, 2005; Roman et al., 2006). Leal et al. (2006) have also used lipases in the enzymatic treatment of dairy wastewater. In contrast to restaurant wastes which have a uniquely high lipid content, general aerobic and anaerobic wastewater treatments are expected to contain a major organic fraction especially rich in protein and carbohydrate content (Goel et al., 1998b). Proteases and glycosidases are therefore believed to play a pivotal role in the degradation of wastewater sludges.

Composition of wastewater treatment systems – the key role of metabolic intermediates, substrates and products

Indeed, the suite of enzymes required may change and depend greatly on the composition of the wastewater influent. In addition, these enzyme activities may also change depending on the level of metabolic intermediates present in the treatment system. For example, Whiteley et al. (2002b; 2003b; 2004), and Watson et al. (2004) have shown that the activities of ß-glucosidases and proteases in an anaerobic sulphidogenic bioreactor were stimulated by specific sulphur metabolites (e.g. sulphide: see Fig. 1), while these enzymes were inhibited by high levels of sulphate. Similarly, lipase activities in a standard rate anaerobic digester were also enhanced in the presence of sulphide and sulphite, and

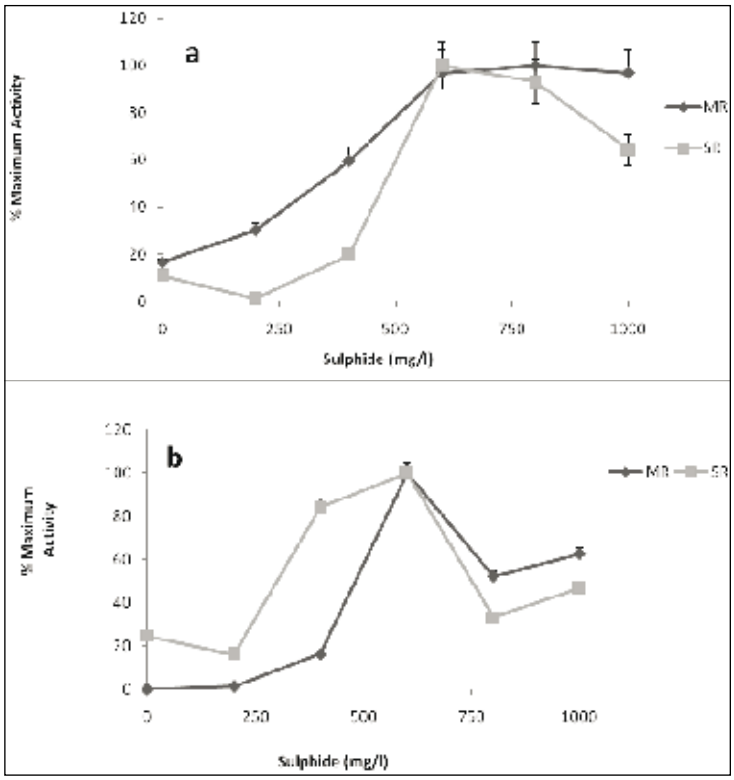

Figure 1
The effect of sulphide concentration on a) β-glucosidase and b) protease activities in a methanogenic bioreactor (MR) and sulphidogenic bioreactor (SR)

inhibited by sulphate (Whiteley et al., 2003a; 2003b). The levels of substrate(s) and product(s) are also important. In enzymology, it is common for enzymes to be inhibited by their products, or to be stimulated by the presence of their substrates (substrate inhibition can; however, also occur at very high substrate concentrations). For example, the addition of hydrolysed starch was reflected by an increase in the α-glucosidase activity of activated sludge (Nybroe et al., 1992). Furthermore, Frølund et al. (1995) have pointed to the fact that the presence of humic compounds in activated sludge may affect enzymatic activity. Also, it is possible for a metabolite to stimulate the activity of one enzyme while exerting an inhibitory effect on another. For example, Watson and Pletschke (2006) have shown that sulphide is able to stimulate the activity of β-glucosidases (a key hydrolase in the digestion of cellulose) while inhibiting the activity of α-glucosidase (a key hydrolase in starch hydrolysis). On a genetic level, the gene expression of enzymes may also be related to the levels of substrates, products, and the levels of associated co-enzymes, cofactors or other metabolic intermediates in the microenvironment.

Designing wastewater treatment by running simulation models, which do not take enzyme expression and induction into account, leads to the model deviating from the real results and the consequence is that design engineers observe different results in the built process unit from those predicted by their pre-commissioning design calculations. To counteract this, biochemical models have been designed to incorporate enzyme induction and repression and metabolic changes. Zhang et al. (2002) proposed a model in which the protein synthesis system grows and decays, depending on the availability of the substrate. The growth rate of the sludge is then dependent on the rate of cell synthesis as determined by protein generation, and hence on the protein synthesis system. This link between biomass growth and substrate utilisation is very well known, but the way in which biological work is presented is not always accessible to the wastewater practitioner.

The production of new genetic material (ribosome synthesis) controls the growth rate of the bacteria in the sludge, and it is related to the **availability** (as differentiated from the **concentration**) of the substrate. It has been stated that kinetic parameters lumped together under the broad term 'endogenous decay' are in fact a wide range of cell biochemical reactions, such as endogenous respiration, maintenance energy requirements, cell lysis and decay and the effects of toxin or other deleterious physicochemical environmental aspects (Van Loosdrecht and Henze, 1999). Therefore, straightforward translation of oxygen uptake into substrate degradation is incorrect, as the rates of hydrolysis and oxidation are affected by substrate being enmeshed with the biomass, the storage of substrate products and the presence of slowly biodegradable COD (Gujer et al., 2000).

Cybernetic models have also been proposed to take enzyme production and activity into account (Lavallée et al., 2002). Cybernetic models simulate enzyme production and activation in bacteria in order to model the gain and loss of parts of the protein synthesis system; this was given the name 'resource machinery'. Cybernetic models include mathematical modelling of enzyme induction mechanisms as well as kinetic functions such as growth and respiration rates. Hence these models link enzyme synthesis and activity to the substrate available and have been shown to simulate chemostat cultures well. The impact of enzyme induction in denitrification in activated sludge was demonstrated by Lee et al. (2004), who used a cybernetic model proposed six years earlier (Liu et al., 1998). However, even this model only included one component to simulate short-term enzyme synthesis, and to include longer term protein synthesis (such as genetic material). Another, longer term time component must also be incorporated (Lavallée et al., 2002).

The role of extracellular polymeric substances

Several reports have suggested that the hydrolases are mainly localised in the extracellular polymeric substances (EPS) matrix of bacterial aggregates (Frølund et al., 1995; Guellil et al., 2001). The EPS originate from bacterial active secretion (Wawrzynczyk et al., 2007b) and from debris present in the sewage sludge itself. This debris can be either organic or inorganic (Tchobanoglous et al., 2003). Extracellular polymeric substances are composed of a variety of organic substances such as carbohydrates, proteins, humic compounds, lipids, uronic acids and deoxyribonucleic acids (Tchobanoglous et al., 2003). It is uncertain as to whether or not the EPS matrix assists or hinders the hydrolysis step. Tchobanoglous et al. (2003) have stated that EPS, together with multivalent ions, aid the formation and settling of sludge flocs in both aerobic and anaerobic sludge treatment systems. In contrast, Tchobanoglous et al. (2003) have also stated that an excess of EPS may hinder the dewatering of sludge, bio-flocculation and sludge settling (Liu and Fang, 2003; Tchobanoglous et al. 2003). Cadoret et al. (2002) stated that the diffusion of substrates in activated sludge flocs, and their subsequent availability to the extracellular enzymes may be hindered by this matrix. Cadoret and co-workers found that approximately 17% of L-Leu-aminopeptidase, 5% of alpha-glucosidase, 23% of protease and 44% of alpha-amylase activities were associated with the extracted EPS component of the flocs. In their studies, they examined the extent to which the diffusion of high molecular weight compounds through the EPS matrix in activated sludge aggregates reduced their availability to the extracellular enzymes (ectoenzymes and exoenzymes). The rate of amylose (azure) hydrolysis increased five-fold when the activated sludge flocs were dispersed by ultrasound and a cation exchange resin, indicating that amylose hydrolysis was indeed hampered by the presence of the EPS matrix. However, no change in the rate of protein (azocasein) hydrolysis was observed when the EPS matrix was dispersed. It appears therefore, from the work performed by Cadoret and co-workers, that the EPS matrix may hinder the accessibility of the substrates to the enzymes. In contrast to this observation, Ayol (2005), Frølund et al. (1995) and Vavilin et al. (1996) stated that the EPS may act as a trapping network that serves to confine the extracellular hydrolases, i.e. act as a sink for the immobilised enzymes (Frølund et al., 1995).

In order to study the hydrolases in wastewater sludges, the EPS matrix usually has to be dispersed in order to obtain and further purify the enzymes. This has been achieved using cation exchange resins (CER) alone, or in combination with the non-ionic detergent Triton X-100 (Gessesse et al., 2003). Triton X-100 has also been used in combination with EDTA (ethylenediaminetetraacetic acid) for the extraction of lipases and proteases from activated sludge samples (Gessesse et al., 2003).

The effects of various sludge pre-treatments

Heat treatment has been used by Yan et al. (2008) for the reduction of excess sludge. This is a relatively simple process and the relationship between the efficiency of sludge reduction and biological response of the sludge matrix was investigated using microbial population and protease activity. Protease-secreting bacteria emerged shortly after heat treatment, with an instant

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increase in protease activity in the sludge supernatant after 1 h heat treatment. This protease activity was activity believed to have been released from the microbial cells via lysis (Yan et al., 2008).

Ultrasonic pre-treatment of sludge has also been used (Yu et al., 2008). Ultrasonic treatment can improve the aerobic digestibility of sludge, and therefore lead to enhanced sludge reduction. Yu and co-workers reported that ultrasonic pre-treatment enhanced the activities of various enzymes and promoted the shift of extracellular proteins, carbohydrates and enzymes from the inner layers of sludge flocs to the outer layers, leading to increased contact and interaction between these components and higher efficiencies in aerobic digestion. They also showed that ultrasonication effectively extracted the EPS from the sludge flocs, and that there existed no correlation between the biochemical composition of the EPS and the distribution of enzymes (such as proteases, alpha-amylases, alpha-glucosidases) within the sludge matrix (Yu et al., 2007).

Cation-binding agents such as sodium tripolyphosphate (STPP), citric acid (CA) or ethylenediaminetetraacetic acid (EDTA) have been used to improve the solubilisation of sludge (Wawrzynczyk et al., 2008). The cation-binding agents are believed to disrupt the adsorption of enzymes to the sludge matrix via polyvalent metal ions, thereby liberating the trapped or bound enzymes from the sludge structure. The increased availability of enzymes is believed to stimulate a more efficient release of organic matter from the sludge. Wawrzynczyk et al. (2007a) also investigated the effects of the cation-binding agents above (and others such as formic acid, citric acid, tartaric acid, Zeolite A, sodium fluoride, sodium thiosulphate and sodium silicate) on the solubilisation of municipal sludge and sludge structure (alone or in combination with enzymes-the treatment of the sludge with cation-binding agents was followed by the addition of three glycosidic enzymes). They reported that, once again, the use of the cation-binding agents above resulted in higher stability of the added enzymes and improved sludge digestion.

As mentioned previously, a number of authors have shown the benefit of enzyme addition or pre-treatment on the conditioning of wastewater solids (primary sewage sludges) and enhancement of the degree of dewaterability of anaerobically digested biosolids (Ayol, 2005; Ayol and Dentel, 2005; Roman et al., 2006). Kim and Sim (2004) optimised sludge pre-treatment in their study by controlling the amount of enzyme and ozone. Dursun et al. (2006) reported that there was a significant increase in cake solid content of anaerobically digested sludge (27% as opposed to 18% without enzyme pre-treatment) using an enzyme dose of 20 mg/ℓ.

Sesay et al. (2006) also investigated enzyme hydrolysis as a mild and effective means of extracting extracellular polymers from mixed culture activated sludge flocs. Alpha-amylase, cellulase and proteinase were used in this study. Enzymatic extraction of the extracellular polymers was found to be quite rapid and only required a few hours. No significant cell lysis was observed. Proteins and carbohydrate components of the EPS were found to co-extract, indicating that these two components existed bound to each other in the sludge matrix. This enzyme extraction method, however, if compared to the traditional cation exchange resin method, generally results in a lower estimate of polymer content (Sesay et al., 2006).

Different electron acceptor conditions

Activated Sludge Model No. 2 recommends hydrolysis rates under anaerobic and anoxic conditions as 10% and 60% of the aerobic hydrolysis rates, respectively. There is a lack of consensus in literature regarding the rates of hydrolysis under different electron acceptor conditions. Goel et al. (1998a; 1998b) found that the effect of electron acceptor conditions on the hydrolases was significant for pure cultures (*Bacillus amyloliquefaciens* or *Pseudomonas saccharophila*), but only marginal for activated sludge. The activities of the hydrolases under the aerobic and anaerobic phases of a sequencing batch reactor were found to be roughly of the same magnitude, contradicting the assumptions of lower hydrolytic rates under anaerobic and anoxic conditions. Goel and co-workers have proposed that the enzymes that are floc-bound are recycled in single sludge systems and that steady-state (equilibrium) is established between enzyme synthesis and loss. They then studied the activities of four hydrolases (alkaline phosphatase, acid phosphatase, alpha-glucosidase and protease) under different electron acceptor conditions. These enzyme activities were each uniquely dependent on the following key considerations: rate of enzyme synthesis, stability of the enzymes involved and the location of the enzymes in the sludge. Goel et al. (1998b) concluded that these enzyme properties should be considered along with the treatment process layout in order to establish the reduction factors under different electron acceptor conditions.

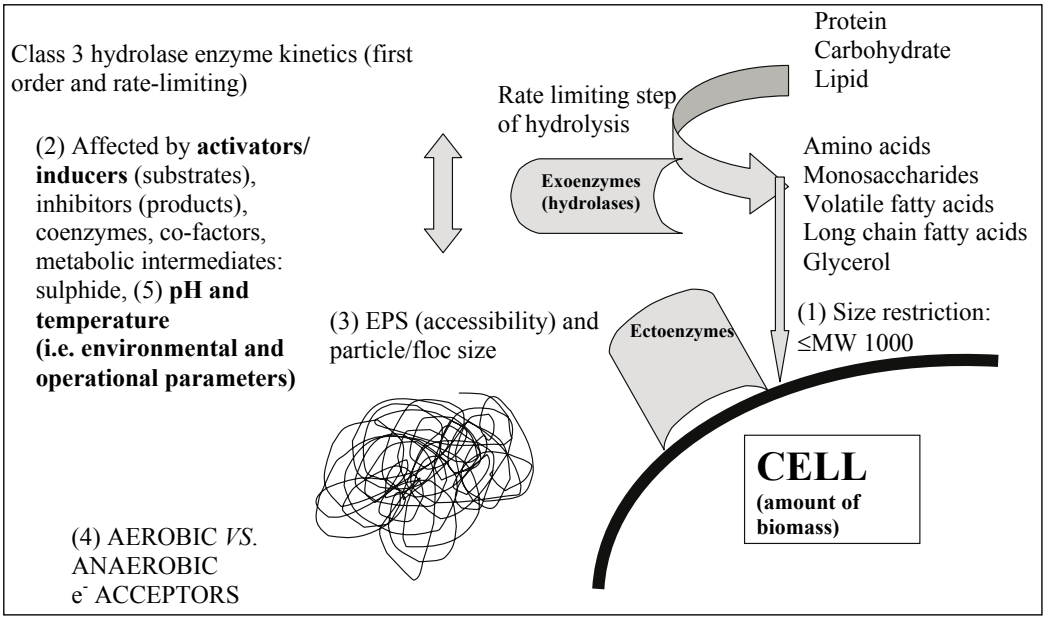
Temperature and pH

A great deal of effort is expended to maintain set conditions within process units. The operating pH range of most units is controlled in the range 6.5 to 8.5, depending on the wastewater and the target pollutant. There is no such thing as an overall ideal operational pH, since all processes employ consortia of micro-organisms, whose growth rates at different pH values are not the same. In addition, each species of micro-organism will use several different enzymes in its metabolic processes, and all of these also have varying pH optima. Hence the operational pH is a compromise, a value which can be tolerated by all enzymes and micro-organisms involved, but with a proportion of them showing activity at suboptimal level. The pH within sludge can change according to a range of processes which impact upon it: CO_2 production (from aerobic respiration, growth and endogenous activity), CO_2 removal (stripping via aeration in aerobic processes or surface stripping in anaerobic processes), uptake of ammonium or nitrate for growth or as an electron acceptor, and uptake of weakly acidic substrates for growth (e.g. acetate). Utilisation of substrates such as acetate consumes protons because the biomass maintains its cellular level charge (Gernaey et al., 2002). Conversely, the uptake of substrates such as dextrose, which are present in undissociated form, does not consume protons. The prevailing pH exerts an effect on almost all cellular processes: substrate uptake and storage, cell growth, degradation of storage products and endogenous processes.

Similarly, temperature influences almost all cellular reactions. In general, reactions proceed at faster rates under higher temperatures, but all enzymes have temperature optima and tolerance ranges below and above which substrate utilisation is slowed. The temperature effect can be modelled using the modified Arrhenius equation (Sin, 2004):

$$r(T) = r(20^{\circ}C) \cdot \theta^{\cdot(T-20)} \quad (1)$$

Where the temperature correction coefficient, θ, can be found from the literature or determined experimentally for each biological process under consideration.



Scheme 1
Physico-chemical and enzymatic components of the rate-limiting step of anaerobic digestion- hydrolysis. Mass transfer limitation (1), metabolic intermediates (2), EPS (3), electron acceptor conditions (4) and pH and temperature (5) all have a dramatic effect on the activity of the enzymes involved in wastewater treatment.

In contrast to the importance assigned to temperature control in biological / enzymological studies, wastewater treatment plant process designs are lumped into two very broad temperature categories: mesophilic and thermophilic. These two categories refer to broad ranges of operating temperatures, 20 to 40°C for mesophilic and 45 to 65°C for thermophilic operation. The temperature is important in determining the rates of reactions, especially hydrolysis and methane generation (for anaerobic units), but in practice temperature control may be only as precise as plus or minus one whole degree. Since a change of 10°C can approximately halve or double a reaction rate, or inhibit it completely, the permitted variability can have profound effects on process performance.

Conclusions

From an enzymatic point of view, aerobic and anaerobic processes are each characterised by groups of micro-organisms and their associated enzymes (many micro-organisms are facultative and can be active in both the environments; in this case, the hydrolytic enzymes are identical). Enzymes secreted or associated with these micro-organisms (especially the Class 3 hydrolases) are vital for the rate-limiting step of anaerobic digestion, hydrolysis (see Scheme 1). The activities of these hydrolases hold the key for establishing the overall limiting rate of the processes, and this rate limiting step involves the breakdown of large polymeric substrates such as carbohydrates (cellulose, starch, proteins) into small low molecular compounds (glucose, amino acids, etc.) less than 1 000 Daltons in size which are able to enter the bacterial cell and participate in its cellular metabolism (see Scheme 1). In aerobic treatment processes, these hydrolases are usually free in the medium or contained within an EPS matrix. In the case of anaerobic treatment processes, these enzymes may be free in the extracellular environment, or in the case of carbohydrate degrading enzymes (glycohydrolases) contained and immobilised within a large multi-enzyme containing catalytic complex called a cellulosome (only present in some bacteria). Enzymes are often more stable when immobilised onto solid media such as substrate, EPS and floc particles, and the physical effect of these components on the enzyme can also not be ignored (Scheme 1).

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