Hydrolysis kinetics of dissolved polymer substrates

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Abstract In this paper, the relation between the hydrolysis rate of dissolved polymer substrates and sludge concentration was investigated in two ways, viz. by laboratory experiments and by computer simulations. In the simulations, the hydrolysis of dissolved polymer components was regarded as a general depolymerisation process in which the bonds of the parent molecule break randomly until only monomer and dimer components remain. The results illustrate that for the hydrolysis of dissolved polymer substrates the enzyme activity is the rate-limiting factor. Moreover, a general depolymerisation process can describe the enzymatic hydrolysis of these components.

Keywords Anaerobic digestion; complex substrates; depolymerisation; dissolved polymers; hydrolysis; model

Introduction
During the anaerobic digestion of complex waste(water) the hydrolysis is the first and often the rate-limiting step. In the hydrolysis stage, polymeric compounds are converted by extra-cellular enzymes to soluble mono- or dimers that are readily available to the acidogenic bacteria. The polymeric components in waste(water) that need to be hydrolysed can be found in different physical states, in particles, dissolved or emulsified. Particles are the most commonly found. Significant amounts of dissolved polymers can be found in slaughterhouse wastewater (gelatine) or potato processing wastewater (dissolved starch). Emulsified polymers are usually lipids, for instance those prevailing in olive mill effluents. Sanders et al. (2000) showed that the hydrolysis rate of particulate substrates is limited by the amount of surface available to the hydrolytic enzymes. When dealing with dissolved substrates the available amount of surface is much larger and corresponds to the total amount of substrate. Therefore it is very likely that in the case of dissolved polymer substrates the amount of active enzymes plays a role. As research dealing with the kinetics of the hydrolysis of dissolved compounds has indicated that the hydrolytic enzymes are located on the sludge (Goel et al., 1998; Confer and Logan, 1998a) the sludge concentration could affect the hydrolysis rate of dissolved polymer components.

In this paper, the relation between the hydrolysis rate and sludge concentration was investigated in two ways, viz. by laboratory experiments and by computer simulations. In the simulations, the hydrolysis of dissolved polymer components was regarded as a general depolymerisation process (Montroll and Shima, 1940; Ziff and McGrady, 1986; Kostouglo, 2000). This approach not only allowed for investigation into the effect of the enzyme activity, but also into the occurrence of polymer fragments during the degradation process as was observed by Confer and Logan (1997a,b, 1998a).

Methods
Lab experiments. Goel et al. (1998) carried out research into the relation between the hydrolysis rate, substrate concentration and biomass concentration by using batch experiments. In the research presented here similar experiments were carried out, but with a protein, gelatine (Merck, PA), as a substrate. The sludge used in the experiments was fresh
waste activated sludge taken from a pilot plant. This pilot plant was fed with domestic wastewater at a loading rate of 0.1 kg COD/kg TSS/day. The relation between the initial substrate concentration and the hydrolysis rate was assessed in 250 ml serum-flasks all filled with 10 g TSS/l of fresh activated sludge. The initial gelatine concentration in the serum-flasks ranged from 1 to 4 g gelatine/l. The effect of the sludge concentration on the hydrolysis rate was determined in a second experiment, using activated sludge diluted with demi-water to make a concentration range between 3 and 11 g/l TSS. At the start of the experiment a gelatine stock solution was added to a final concentration of 2 g/l gelatine. Both experiments were performed in duplicate. After filling the flasks they were flushed with N2 for 3 minutes and incubated at 30°C on a wrist action shaker. Paper filtered samples (Schleicher and Schuell 595) were taken 2.4, 3.3 and 6.0 hours after incubation and immediately after sampling the protein concentration of the samples was determined by Biuret method (Herbert et al., 1971) with gelatine as standard. The initial hydrolysis rate was calculated according to Eq. (1).

\[
\text{initial hydrolysis rate} = \frac{(S_0 - S_1)}{\Delta t}
\]

With:
- \( S_0 \) = gelatine concentration at the start of the experiment (g/l),
- \( S_1 \) = gelatine concentration in the first sample that is taken from the serum-flask (g/l),
- \( \Delta t \) = amount of time between the start of the experiment and the time at which sample \( S_1 \) is taken (h).

The depolymerisation model. As mentioned earlier several authors have derived equations to describe the progress and chain length distribution during the depolymerisation of polymers. In this paper the model presented by Montroll and Shima (1940) was used. In their theory Montroll and Shima (1940) made the following assumptions.

1. All molecules originally have the same molecular weight.
2. The accessibility to reaction of a bond in a given chain is independent of the position in the chain and independent of the length of its parent chain. Thus, bonds break randomly irrespective of their position in the chain.
3. All chains in the mixture are equally accessible to reaction.

By applying statistical calculations they derived the following equations for the fraction of monomers present in chains with a length \( q \) when an average number of cuts \( (r_0) \) has been made to each macromolecule with a length of \( p+1 \) monomers.

\[
\Phi_q(p, \alpha) = \frac{\alpha q (1-\alpha)^{q-1}}{(p+1)} [2 + (p-q)\alpha] \quad q \leq p, \alpha \leq 1
\]

(2)

\[
\Phi_{p+1}(p, \alpha) = (1-\alpha)^p
\]

(3)

\[
\alpha = \frac{r_0}{p} \quad \alpha \leq 1
\]

(4)

With:
- \( p \) = number of bonds in the parent macromolecule,
- \( \alpha \) = average degree of polymerization, \( \in [0;1] \),
- \( r_0 \) = average number of scissions made to a macromolecule,
- \( q \) = number of monomers in the macromolecule under consideration,
\( \Phi_q \) = fraction of monomers involved in chains with a length of \( q \) monomers,
\( \Phi_{p+1} \) = fraction of monomers involved in chains with a length \( p+1 \).

To describe the hydrolysis of dissolved polymers during anaerobic digestion some adaptations were made to the model of Montroll and Shima (1940).

1. In this paper hydrolysis is defined as the conversion of polymer substrates to merely monomer and dimer components which implies that the hydrolysed fraction (\( \Phi_{\text{hydr}} \)) can be calculated by the sum of \( \Phi(p, a) \) at \( q = 1 \) and \( q = 2 \).

2. Montroll and Shima assumed that all polymers have the same initial chain length. However, in wastewater the initial length of the molecules varies over a certain range. The evolution in the chain lengths of a distribution with molecules between 3 and \( n \) monomers can be calculated by the sum of each separate chain length. Moreover, each chain length has to be multiplied by the factor \( n_p \), comprising the fraction that each chain length contributes to the total distribution range (Eq. (5)). The hydrolysed fraction is calculated in a similar way (Eq. (6)).

3. To introduce the aspect of time it is assumed that the enzymes executing the hydrolysis have a constant activity (E). This activity can be expressed as the number of scissions to each parent macromolecule per unit of time and is similar to \( V \) (or \( dS/dt \)) in the Michaelis–Menten kinetics (Eq. (8)). Eqs (5) to (8) are hereafter in this paper referred to as the depolymerisation model.

\[
\Phi_{q,\text{distribution}}(p, \alpha) = \sum_{p=3}^{p=n} n_p \frac{\alpha q(1-\alpha)^{q-1}}{(p+1)} [2 + (p-q)\alpha] \quad q \leq p, \alpha \leq 1
\]  
(5)

\[
\Phi_{\text{hydr},\text{distribution}}(p, \alpha) = \sum_{p=3}^{p=n} n_p \frac{2\alpha + \alpha^2(p-1) + 4\alpha(1-\alpha) + 2\alpha^2(p-2)(1-\alpha)}{(p+1)} \quad \alpha \leq 1
\]  
(6)

\[
n_p = \frac{\text{no. of monomers in chains with length } p+1}{\text{total no. of monomers in distribution range}}
\]  
(7)

\[
\alpha = \frac{E \cdot t}{p} \quad \alpha \leq 1
\]  
(8)

with:

\( E \) = enzyme activity (scissions per parent macromolecule(spm)/h),
\( t \) = time (h).

The substrate for the model calculations. To simulate the hydrolysis of a wastewater containing polymer components, with Eqs (5) to (8) a substrate with a well defined polymer size distribution is required. For the model simulations, it was therefore assumed that the model substrate resembled dextran 70 k (Confer and Logan, 1997b; Carlson and Silverstein, 1998). A distribution was calculated based on the following assumptions (Table 1):

- 1.0 k is approximately 7 monomeric units (Confer and Logan, 1997b);
- the chain length distribution resembles a Normal Distribution;
- the dissolved organic carbon (DOC) is distributed as depicted by Carlson and Silverstein (1998);
- chains smaller than 0.5 k are considered to be hydrolysed;
- chain lengths are packed together in groups at intervals of 10 monomeric units.

The results of the simulations were divided in three fractions to allow comparison with the results from the laboratory experiments and those obtained by Confer and Logan (1997a,b).
1. Hydrolysed components $\Phi_{\text{hydr}}$, the fraction of monomer components present in chains of length 1–2 monomer units.

2. Intermediate size components $\Phi_{7–70}$, the fraction of monomer components present in chains of length 7–70 monomer units or 1–10 k (Confer and Logan, 1997b).

3. Small size components $\Phi_{3–6}$, which is the fraction of monomer components present in chains of length 3–6 monomer units or 0.5–1 k.

**Results and discussion**

**Accumulation of hydrolysis intermediates.** Confer and Logan (1997a,b) reported accumulation of hydrolysis intermediates during the degradation of dextran, dextrin and bovine serum albumin. Especially an increase of components smaller than 1 k (1–6 monomers) was observed. The changes in the intermediate size fraction (7–70 k) were only small. The latter was attributed to the fact that the substrate already contained a significant amount of intermediate components. Figure 1 reveals the accumulation of small size (3–6 monomers) and intermediates size (7–70 monomers) hydrolysis intermediates as they appeared from simulations with the depolymerisation model. The figures show accumulation of intermediate and small size components at both enzyme conditions. The maximum fractional amounts that accumulate are 0.736 and 0.322 for the intermediate and small size fraction, respectively. Although the height of the peaks is equal for both conditions the maximum is reached within a shorter time at higher enzyme activity. Moreover, the small size fraction remains detectable longer than the intermediate size.

**The relation between the hydrolysis rate and the sludge concentration.** Goel et al. (1998) investigated the hydrolysis rate of dissolved starch at several sludge and substrate concen-
trations in a batch experiment. From the results obtained, it appeared that the initial hydrolysis rate was linear related to the sludge concentration ($R^2 = 0.9288$). The relationship found between the initial hydrolysis rate and the substrate concentration is of a saturation type, in which the inverse initial rate was linear to the inverse substrate concentration ($R^2 = 0.9998$). Goel et al. (1998) proposed a Michaelis–Menten type of equation for the hydrolysis of dissolved polymers. The results of the here presented research reveal that similar relations prevail for the hydrolysis of gelatine (Figure 2A,B) and the results from the simulations with the depolymerisation model (Figure 2C,D). Although in the case of the depolymerisation model the inverse hydrolysis rate is not linearly related to the inverse sludge concentration but to the inverse enzyme activity (1/$E$). However, as the enzymes that perform the hydrolysis are attached to the cells (Confer and Logan, 1998a; Goel et al., 1998), the enzyme activity will be directly related to the sludge concentration. Consequently the inverse initial hydrolysis rate calculated with the depolymerisation model is also linearly related to the inverse sludge concentration. The depolymerisation model is therefore capable of describing the relationship between the initial hydrolysis rate and the sludge concentration as it was assessed in the lab experiments.

**Conclusions**

Results of the present batch experiments with starch (Goel et al., 1998) and gelatine (this paper) reveal that for the hydrolysis of dissolved polymer components: the initial hydrolysis rate and sludge concentration are linearly related; and the inverse initial hydrolysis rate and inverse initial substrate concentration are linearly related. From these results it is clearly illustrated that unlike particulate substrate, the hydrolysis of dissolved polymers is limited by the enzyme activity. Moreover, it is shown that the mechanism of the enzymatic hydrolysis can be described by a random depolymerisation process.

![Figure 1](https://iwaponline.com/wst/article-pdf/45/10/99/425010/99.pdf)  
**Figure 1** Simulation of the hydrolysis of the model compound by the depolymerisation model at $E = 1$ spm/h (upper graph) and $E = 5$ spm/h.
References


