Effects of initial molecular weight on removal rate of dextran in biofilms

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Abstract

Degradation kinetics of different size dextrans in a biofilm reactor were evaluated. Degradation rates of dextran standards, measured as time series of oxygen utilisation rates (OUR), decreased with increasing initial molecular weight. Removal of bulk phase TOC with time was highly correlated ($R^2 > 0.99$) and could be modeled with a variable half order degradation rate expressions. A power correlation between initial molecular weight and the variable half order degradation rate coefficient was found for polymers in the range 6-500 kDa. Degradation of dextran in the colloid size range ($M_W > 1$ MDa) did not follow the same kinetics. Reductions in the observed removal rate with polymer size can be explained by the effect of reduced diffusivities of the substrate, without assuming reaction rate effects. This suggests that removal of polymers and particles in biofilm systems is transport limited.

Keywords: Dextran, biofilm, diffusion, half-order kinetics, hydrolysis

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Introduction

More than 95 % of the available organic matter in natural aquatic environments is composed of high molecular weight compounds (Münster and Chróst, 1990). In municipal wastewater a significant fraction of biological oxygen demand is found as suspended organic particles and dissolved polymeric material (Levine et al., 1985). Direct bacterial uptake of substrates from the surrounding media is limited to molecules of molecular weight (M_W) less than 600-1000 Da, thus degradation of macromolecules (here defined by that limit) depends on extracellular enzymatic depolymerisation followed by uptake and mineralisation (Chróst, 1991; Confer and Logan, 1998a; White, 2000). In the wastewater engineering literature degradation of macromolecules and particulate organic matter is often lumped into the term hydrolysis. A quantitative understanding of hydrolysis kinetics is essential for the understanding of local electron donor utilisation rates, nutrient removal performance and microbial population dynamics as discussed in a review by Morgenroth et al. (2002). Even though hydrolysis is regarded as a central process in wastewater systems, uncertainties still prevail regarding mechanisms (stoichiometry) and rate (kinetics) relations (Henze et al., 2000).

Early models of particulate substrate degradation were based on direct growth (Stenstrom, 1975) or adsorption followed by direct growth (Ekama and Marais, 1979; Dold, 1980; Frigon et al. 2001). Hydrolysis of slowly biodegradable into easily biodegradable substrates was adopted by the IAWQ task group on mathematical modeling for design and operation of biological wastewater treatment processes, as a one step hydrolysis process (Henze, et al., 1987). Several authors have expanded this lumped substrate model to separately describe the kinetics of slowly, intermediate, and rapidly hydrolysable substrates in order to reflect the chemical heterogeneity and molecular weights of particulate substrates. Sollfrank and Gujer (1991), Orhon et al., (1998), Janning, (1998), and Vollertsen and Hvidtved-Jacobsen (1999) defined parallel hydrolysis into easily biodegradable substrates, while Novak et al. (1995),

Bjerre (1996), Confer and Logan (1997) and Spérandio and Paul (2000) applied sequential hydrolysis. Separation into substrate classes based on degradability reflects the complexity of particle and polymeric substances degradation.

Models and parameters from studies of suspended systems (e.g. Henze and Mladenovski, 1991) may not be directly applicable in biofilm systems where there is less biomass-liquid interface area per biomass, causing less efficient mass transfer from the bulk liquid to the biomass in the biofilm. Hydrolysis of particulate and colloidal organic matter in biofilm systems has been studied by several groups (Sprouse and Rittman, 1990; Larsen and Harremoës, 1994; Janning et al., 1998; Janning, 1998). Confer and Logan (1997a,b; 1998a) studied depolymerisation of model polymeric substrates in biofilm systems and suspended cultures. Using the polysaccharide dextran, they showed how intermediates may form, and discussed how intermediate formation and hydrolysis are effected by mass transfer limitations in biofilms and other bioaggregates with diffusion gradients.

Kinetics of biodegradation in biofilm systems have been mainly evaluated using dissolved substrates. The behaviour of readily diffusive low molecular weight compounds like acetate and glucose in biofilms has successfully been described by a set of diffusion-reaction equations (Harremoës, 1978). However, mass transfer in gel like structures like biofilms may be restricted by physical size. Even though large polymers, or particles, may not be able to penetrate into the biofilm matrix, these molecules are thought to adsorb to the biofilm surface (Bouwer, 1987; Guiot et al., 2002; Thurnheer et al., 2003). Following biofilm surface deposition, surface extracellular enzymes would act upon adsorbed polymers and particles, and release smaller intermediates, which would be able to penetrate into the biofilm for further depolymerisation and oxidation (Haldane and Logan, 1994; Confer and Logan, 1998b). Based on this conceptual model, the molecular weight and the geometry of a polymeric substrate will influence degradation kinetics due to possible sorption effects,

diffusion restrictions and mechanisms of depolymerisation. A mathematical description of polymer degradation must take into account all of these potentially limiting processes.

In this paper we investigate how the initial molecular weight of an added model substrate affects observed degradation rates in a mixed population batch operated biofilm reactor. Batch experiments were performed with different size dextrans and observed degradation rates were evaluated using an analytical solution of a diffusion-reaction model.

Material and methods

The experimental set up is presented in Figure 1. An LJ 1120 model rototorque biofilm reactor (Biosurface Technologies, Bozeman MT, USA) was equipped with an external recirculation loop (Watson-Marlow 313 U pump at approx. 500 ml/min) providing online pH and oxygen control, and a counter current bubble column for re-oxygenation of the reactor bulk liquid. The total reactor system volume was 1160 ml, with a total wetted surface area of 0.2890 m^2, giving a specific biofilm area of 250 m^2/m^3. The bubble column was attached to a debubbler through a narrow tube connection to minimize diffusive backward re-oxygenation. Bulk phase mixing and biofilm surface shear conditions were kept constant by operating with a fixed rotor speed (210 rpm) during normal operation. Liquid phase dissolved oxygen concentrations (DO) were monitored using a WTW Oxi 340A meter with an Ox 325 probe placed in a flow through mixing cell with magnetic stirring. An additional flow cell was used for online pH measurements using a Hanna HI 1910B probe with a Metrohm 692 meter. Both signals were logged using custom made software (LabVIEW 6.0e, National Instruments, Austin TX, USA) on a desktop PC with an Advantech PCL 818L (Advantech Co. Ltd, Taiwan) data acquisition card. Oxygen and pH control was provided by automatic operation of a solenoid valve for pure oxygen addition, and control of a pump (Ismatech Mini-S 860) connected to a 2 M NaOH reservoir. During continuous operation, computer controlled

operation of mineral salt solution/substrate and dilution water pumps (Ismatech Reglo Analog MS4/6-100 and Ole Dich Digital, respectively) provided controlled loading through a drop chamber for back-growth prevention. Temperature control was established using an external water bath (Hetofrig) with local temperature control (25°C), recirculated (at approx. 500 ml/min) through the outer cylinder of the jacketed LJ 1120 reactor.

Model substrate. Dextrans are a class of macromolecules synthesised by some bacteria and fungi, composed of α (1-6)-D-glucopyranosyl units. 3-4% of glucose units in dextran carry a third glucoside bond at either α (1-2), α (1-3), or α (1-4) (Lehninger et al. 2000). In this study, dextrans of various M_W from the lactic acid bacterium *Leuconostoc mesenteroides* strain B-512F were obtained from Sigma-Aldrich, MO, USA. Based on product information (Sigma-Aldrich), standards were normal distributed to the logarithm of the average molecular weight (Kuhn distributed; Smidsrøed and Moe, 1995). Dextrans of M_W >10 kDa behave as typically branched flexible polymers, while standards of 2-10 kDa exhibit the properties of a random chain/expandable coil and below 2 kDa as stiff chain, rod like molecules (Product information Sigma-Aldrich). These properties are comparable to polymers found in wastewater (proteins and flexible polysaccharides), and dextran should therefore be a good surrogate for mimicking the behaviour of such molecules in biofilm systems.

Culture media. Stock phosphate buffered mineral salt solution (PBS) was diluted by deionised water to the following inlet concentrations: Na_2SO_4 0.05 mM, NH_4Cl 0.9 mM, NaCl 0.5 mM, $MgCl_2$ 0.2 mM, K_2HPO_4 0.04 mM, KH_2PO_4 0.3 mM, $CaCl_2$ 0.05 mM, $MnCl_2$ 4μM, $CuSO_4$ 2 μM, $CoCl_2$ 2 μM, $(NH_4)_6Mo_7O_{24}$ 0.3 μM, $Na_2B_4O_7$ 0.5 μM, $ZnCl_2$ 3 μM, $FeCl_3$ 4 μM. In order to prevent autotrophic growth, allylthiourea was added to a final reactor concentration of 0.2 mM. Stock dextran solutions with average molecular weights of 0.18 (i.e. glucose), 6, 10.5, 41.3, 160, 473, 513 and ~35000 kDa were prepared by dissolving 23 g/l dextran powder of the appropriate M_W in buffer (1.4 mM KH_2PO_4 and 9.5 mM K_2HPO_4).

Culture conditions. The inoculum was derived from biomass sampled from the denitrifying reactor at Hundested WWTP Sjælland, Denmark. The biomass attached to the carrier material Biocarbone^{TM} was placed in a packed bed laboratory reactor as described by Janning (1998), and operated for a period of two months anoxically with high M_W dextran (4 - 50 MDa) as carbon source. After this period of acclimation 5 liters of effluent were collected, and filtered through GF/A (pore size 1.6 µm) filter paper (Whatman, Springfield Mill in Maidstone, Kent, UK) to remove eukaryotic organisms in order to minimize oxygen utilisation and potential phagocytosis of larger dextrans by grazers (Alberts et al., 1994). The permeate was filtered through a 90 mm 0.22 µm cellulose nitrate membrane filter (Whatman prod. no. 7184-009), and the retentate was resuspended in 10 ml PBS. The rototorque reactor used in this study was then inoculated by adding 4 ml of the retentate to the bulk phase containing 500 mg/l dextran (160 kDa) and 100 mg/l Difco yeast extract. Following 30 h of aerobic batch operation, continuous loading of 200 mg/l dextran 160 kDa and 7 mg/l yeast extract was initiated. The retention time was stepwise reduced from 1 h to 20 min. An approximate steady state biofilm was observed by microscopic thickness estimation (Bakke and Olsson, 1986) on removable coupons from the rotor after three weeks. During start up, bulk phase pH and DO was kept at 6.2 and 10-20 mg/l, respectively.

Respiration estimation. Oxygen concentrations were automatically logged every 100 ms. Electronic noise in the logging loop was dampened by averaging the measurements over periods of 5 seconds. This average value was stored and used for respiration rate estimations. The oxygen utilisation rate (OUR) was calculated from a linear regression of oxygen concentrations over time. The number of oxygen measurements used for each linear regression was determined automatically based on the previous respiration rate estimation in order to minimize OUR standard deviation, and at the same time keep the frequency of estimations high enough to detect short-term changes. Thus, at background respiration rates (i.e. ~5 mg $O_2/l \cdot h$) ~50 averaged measurements were used, while at high rates (~25 mg $O_2/l \cdot h$)

15 averaged measurements were used to estimate OUR. Bulk phase oxygen concentrations were controlled by on-off aeration between 22 and 30 mg O_2/l in order to prevent oxygen depletion in the deeper parts of the biofilm.

Experimental procedure. After establishing a relatively constant biofilm thickness of approximately 300 μm, the reactor was first flushed with diluted PBS for 6 hydraulic retention times, and subsequently background respiration was established during batch operation. Upon stable background respiration, 5 ml of a dextran standard were injected, bringing the total bulk phase concentration to 100 mg dextran/l. The reactor was then operated in batch mode. Respiration rates were monitored and bulk phase samples were collected for TOC analysis. Batch operation continued until stable background respiration rates were re-established. Upon batch termination, the reactor was operated for six retention times at minimal residence time (approximately 14 min) and increased shear conditions (430 rpm) to washout remaining bulk phase substrates, and accumulated detached biomass. The procedure also helped keeping the initial biofilm thickness constant prior to each batch experiment. Individual dextran addition experiments were replicated. The selected experimental conditions were tested for reproducibility by repeating experiments for the applied dextran standards at various times during the experimental campaign.

Analytical procedures. Bulk phase samples were drawn from the bubble column by a syringe, and immediately filtered through a 0.45 μm Nylon membrane syringe filter (Millipore, MA, US) into acid washed vials. Each sample was conserved by adding 4 M H_2SO_4 (pH in sample < 1.5) and stored at 2°C. Total organic carbon (TOC) was analysed within three weeks using an IO Analytical Model 700 TOC.

Data analysis. The coupled processes of transport, sorption, hydrolysis, and degradation were described using a single rate expression. Bulk phase dextran concentrations in the biofilm batch system are dictated by the areal removal rate (r_A , [g TOC/m^2·h]),

$$V \cdot \frac{dS_b}{dt} = -r_A \cdot A_f \qquad \text{Eq. 1}$$

where V is the bulk phase reactor volume [m^3], S_b is the polymer bulk phase concentration [g TOC/m^3], and A_f is the total biofilm area. Janning (1998) showed how batch experiments could be interpreted using the half order biofilm kinetics model developed by Harremoës (1978), if a pseudo steady state assumption is justified for the substrate profiles inside the biofilm. Depending on the bulk substrate concentration, the observed areal removal rate would change from 0. order via ½. order to 1. order, according to a shift in the intrinsic biofilm degradation rate from 0. to 1. order kinetics. Assuming the biofilm is at any time partially penetrated, intrinsic Monod kinetics may be used to describe the observed bulk phase substrate concentration using a single equation (Rittmann and McCarty, 2001). This approach is valid for partially penetrated biofilms, and is advantageous when the shift between intrinsic kinetics is hard to define. The areal removal rate (r_A) assuming intrinsic Monod kinetics is given by:

$$r_A = k_{1/2,A} \sqrt{S_b - K_S \cdot \ln\left[\frac{K_S + S_b}{K_S}\right]} \qquad \text{Eq. 2}$$

where $k_{1/2,A}$ is the areal specific removal rate coefficient [$(g/m)^{0.5}/d$] and K_S is the Monod half saturation coefficient [g/m^3]. According to Harremoës and Henze (2002), $k_{1/2,A}$ relates to the biofilm intrinsic reaction rate coefficient as

$$k_{1/2,A} = \sqrt{2 \cdot k_{0,f} \cdot D_f} \qquad \text{Eq. 3}$$

where D_f is the substrate diffusion coefficient inside the biofilm [m^2/d]. $k_{0,f}$ is the zero order intrinsic reaction rate coefficient, and represents the maximum substrate removal rate, defined by:

$$k_{0,f} = \frac{\mu_{max} \cdot X_f}{Y_{X/S}} \qquad \text{Eq. 4}$$

where μ_{max} is the maximum specific growth rate of biofilm biomass [1/d], X_f is the biofilm biomass [g/m^3], and $Y_{X/S}$ is the maximum biomass yield coefficient [g/g]. $k_{0,f}$ may be estimated when D_f is known.

The most common basis for the estimation of liquid diffusion coefficients (D_{aq}) is the Stokes-Einstein equation (Cussler, 1984):

$$D_{aq} = \frac{k_B \cdot T}{6 \cdot \pi \cdot \mu \cdot R_e} \qquad \text{Eq. 5}$$

where k_B is the Boltzman constant [J/K], T is the absolute temperature [K], μ is the solvent viscosity [N·s/m^2] and R_e is the hydrodynamic (equivalent sphere) polymer radius [m]. Dextran is polymerized through three separate bonds, a property resulting in a range of rotational and vibrational states between each monomer unit. Thus, the polymer chain becomes extremely flexible, and takes on the structure of a flexible chain (Smidsrød and Moe, 1995). In addition, regular branching causes the molecules to fold back and form a hydrated sphere. The hydrodynamic radius of this sphere may be represented by the radii of inertia, R_G

$$R_e = \xi \cdot R_G \qquad \text{Eq. 6}$$

where ξ is a size independent constant, determined for a range of dextrans by Lawrence et al. (1994) to be 0.67. Due to the flexible and partial branching of the polymer chain, dextran has a low R_G and relates to the molecular weight as

$$R_G \sim M_W^{0.33 \text{ to } 0.5} \qquad \text{Eq. 7}$$

for a polymer dissolved in water. Based on this theoretical evaluation the diffusion coefficient of dextran is expected to relate to the molecular weight to the power of – 0.5 to – 0.33:

$$D_{aq} \sim M_W^{-(0.33 \text{ to } 0.5)} \quad \text{Eq. 8}$$

The validity of this correlation was evaluated by comparing measured dextran diffusion coefficients from the literature to their corresponding molecular weight (Figure 2). Stewart

(1998) reviewed the effective biofilm diffusivities of high molecular weight compounds, but failed to identify a distinct correlation between diffusion coefficients in the liquid (D_{aq}) and in the biofilm matrix (D_f) as a function of their molecular weight. Therefore a constant correlation between water and biofilm diffusion coefficients was used taking into account biomass density (tortuosity effect) (i.e., $D_{,f} = 0.3 \cdot D_{aq}$) and using liquid phase published diffusion coefficients (Wu, 1993; Lawrence et al, 1994; Smidsrød and Moe, 1995; Bryers and Drummond, 1998).

The value of $k_{0,f}$ was estimated by fitting Eq. 2 and 3 to the polymer bulk phase concentration using $D_f = 0.3\ D_{aq}$. Finally, the effect of M_W on $k_{0,f}$ was evaluated by plotting $k_{1/2,A}$ against M_W, to determine whether reaction or diffusion reactions are rate limiting.

Results

Bulk phase TOC following injection of 44.4 mg TOC/l dextran standards (equivalent to 100 mg dextran/l) is presented in Figure 3. Respiration rates of theses experiments are presented in Figure 4. Background (endogenous) respiration is indicated by the OUR level before injection at t = 0 h. OUR could not be estimated during re-oxygenation, observed in the respirograms as gaps on the OUR curves. This effect was especially pronounced during high respiration rates when more frequent re-oxygenation was required. An immediate response can be seen for all standards upon injection except for the blank (pure PBS standard; not included in the figure). This immediate OUR response indicates that hydrolysis of substrates starts without a time lag with the oxidation of lower molecular weight substrates that may have been present in the standards. A concurrent response of OUR increase and bulk phase TOC removal for low and intermediate weight polymers suggests that sorption for these substrates was negligible. This is not the case for the high M_W dextran standard (35 MDa), where a rapid bulk phase TOC reduction is observed without a detectable OUR response. The highest OUR response resulted from the injection of glucose. Also, final degradation, defined

as the time of which background respiration is re-established, was accomplished first for the glucose standard, increasing gradually for larger initial dextran standard sizes. The maximum OUR response followed a systematic decrease as substrate initial average molecular weight increased.

Table 1 lists the half order areal specific removal coefficients for each initial size standard following linear regression analysis after log transformation, and the correlation coefficients obtained from the fit. K_S was held constant at 1.5 g TOC/m^3 based on literature values (Henze et al., 2000), while $k_{0,f}$ was adjusted by least squares minimization to the observed TOC data. From Figure 3 it can be seen that degradation rates decrease with increasing initial average molecular weight of the dextran substrate. The corresponding half order removal coefficients are plotted against the average molecular weight of the initial substrate in Figure 5, and correlate well ($R^2 = 0.98$) through a power relation ($n = 0.20 \pm 0.01$, linear regression analysis). Depending on the initial average molecular weight there seem to be three distinct dynamics involved in the dextran degradation experiments. Data from 6 to 500 kDa show a systematic reduction in $k_{1/2,A}$ where $k_{1/2,A}$ can be approximated as $2.11\ M_W^{-0.20}$. Results from this region cannot be extrapolated to the low M_W region, as glucose does not require hydrolysis, and removal of glucose will be rate limited by a maximum substrate oxidation rate. The single standard above 1 MDa indicates that the degradation follows a different rate, with distinctively different dynamics as the substrate falls into the colloidal size range (defined as 0.05 - 0.5 μm). The estimated $k_{1/2,A}$ was then used to calculate corresponding OUR from

$$\mathrm{OUR} = 0.76 \frac{\mathrm{g\ O_2}}{\mathrm{g\ TOC}} \cdot r_A \qquad \text{Eq. (9)}$$

where 0.76 g O_2/g TOC is the theoretical stoichiometry between oxygen and substrate utilisation. Results are shown in Figure 4 as solid lines. Equation (2) is based on the assumption of steady state and cannot reproduce an apparent initial lag due to mass transport into the biofilm or hydrolysis. As a result, OUR is overestimated during the initial phases of

every batch. The relaxation back to background respiration levels is, however, relatively well described by this simple direct growth model.

Discussion

The effects of initial substrate M_W on removal rates in biofilms are analysed based on the variable order model (Eq. 1-3). The model is assuming a partially penetrated biofilm and pseudo steady state conditions. An evaluation of these assumptions is required in order to assess the validity of the approach. A parameter β can be defined to estimate biofilm penetration (Henze et al., 2002):

$$\beta = \sqrt{\frac{2 \cdot D_f \cdot S_b}{k_{0,f} \cdot L_f^2}} \qquad \text{Eq. 10}$$

Partial penetration can be expected for β smaller than 1. Using estimated $k_{0,f}$ from Table 1, it can be shown that the glucose maximum penetration depth (at the initial concentration) was 120 μm (penetration depth is defined as $L_f \cdot \beta$; Henze et al, 2002), while the maximum penetration for any of the dextran standards was less than 50 μm. Comparing this with the observed biofilm thickness (approximately 300 μm), it can be seen that the biofilm was partially penetrated by substrate at all times for any of the batch experiments,. The appropriateness of the pseudo steady state assumption can be evaluated by the characteristic times of the system dynamics with characteristics times for diffusion (L_f^2/D_f) evaluation (Crank, 1965, Morgenroth and Wilderer, 1999). Calculated characteristic times for the active biofilm thicknesses (< 50 μm) and diffusion coefficients in the described experiments are in the order of < 2 min., which is rapid relative to the observed experimental dynamics. The assumptions on which Eq. 1-3 are based on are, therefore, valid for the conditions studied.

The observed dextran removal rate is a function of both the reaction rate $k_{0,f}$ and the diffusion coefficient D_f (Eq. 3). Based on the observed rate of substrate removal (Eq. 2) or OUR (Eq. 9) the values for $k_{0,f}$ and D_f are not individually identifiable. However, D_f was independently

estimated using Eq. 5. Then $k_{0,f}$ could be calculated as shown in Table 1. There is no apparent correlation of $k_{0,f}$ and the M_W of the dextran. Calculated $k_{0,f}$ in Table 1 are in the same order as reported in the literature (Henze et al., 2002). Based on this analysis it is concluded that the observed reduction in dextran removal rates with increasing molecule size can be attributed mainly to transport limitations rather than to decreasing depolymerisation and degradation rates.

Reaction rate effects would only be seen if the degradation was limited by available enzymes and/or slow enzymatic depolymerisation. The effect would be reflected by a systematic reduction in $k_{0,f}$ in the applied model, but such a change was not observed (Table 1). The lower rate estimated for glucose indicates that the bulk phase concentration of glucose was not high enough to sustain zero order reaction in the biofilm, as opposed to for the polymers.

Figure 2 shows relations reported in the literature between dextran molecular weights and their observed diffusion coefficient in water. As indicated, the diffusion coefficients relate to the dextran M_W in the middle of the theoretically predicted range (Eq. 7) as $D_f = 2.8 \cdot 10^{-9} M_W^{-0.4}$. In Figure 5 it can be seen that $k_{1/2,A}$ relates to the molecular weight as $\sim M_W^{-0.2}$, exactly as predicted by Eq. 5-7, based on experimentally estimated diffusion coefficients (Figure 2). Sensitivity of $k_{1/2,A}$ towards the estimated relation between dextran biofilm diffusivity and initial M_W is indicated by the dashed lines in Figure 5. They represent the fitted correlations to the initial M_W assuming dextran behaving as perfect compact spheres (i.e. $D_f \sim M_W^{-1/3}$) or a flexible chain ($D_f \sim M_W^{-1/2}$), resulting in a $k_{1/2,A}$ to M_W power relation of –1/6 to –1/4, respectively. Using the average value of the estimated $k_{0,f}$ and the modeled effect of M_W on diffusion (estimation done in Figure 2), $k_{1/2,A}$ is plotted against M_W in Figure 5 (grey line). The predicted relation falls on top of the line obtained after regressing the experimental data (solid black line in Figure 5). This supports the conclusion that the observed effect of increasing molecular weight on the areal removal rate is dominated by dextran biofilm diffusion limitations.

The conclusion was further evaluated by also analyzing the data applying the more commonly used first order hydrolysis rate (Gujer, 1980; Henze and Mladenovski, 1991; Sollfrank and Gujer, 1991; San Pedro et al., 1994; Spanjers and Vanrolleghem, 1995; Janning, 1998; Spérandino and Paul, 2000). The corresponding first order areal removal rate, $k_{1,A}$ shows exactly the same correlation to increasing initial dextran M_W ($k_{1,A} = 0.39 \cdot M_W^{-0.2}$) as for $k_{1/2,A}$ (data not shown). Thus, a change of reaction dynamics does not influence the observed removal rate dynamics, supporting the interpretation that substrate diffusion is the rate determining mechanism during biofilm dextran degradation.

The removal of the large dextran standard of 35,000 kDa cannot be described by the same $k_{1/2,A}$ model as for dextran standards up to 513 kDa (Figure 5). A possible explanation for this shift in kinetic regime can be the complete obstruction of large molecules by the extracellular polymeric structure of the biofilm as observed by Guiot et al. (2002) and Thurnheer et al. (2003). Jimenez et al. (1988) found an upper limit for biofilm diffusion of about 2 MDa, while Tanaka et al. (1984) indicated this limit to be lower (10-100 kDa) studying alginate gels resembling biofilm gel properties. More recently, Stoodley et al. (1997) suggest the biofilm matrix to be microporous, with pore diameters between 50 - 200 Å, corresponding to spherical molecules of 20-200 kDa supporting the concept of an upper limit of substrate that can diffuse into the biofilm. However, Bryers and Drummond (1998) point out that the biofilm matrix is heterogeneous in terms of porosity, and that macropores and channels are common for biofilms, suggesting the matrix pore diameter to vary from micro- to macropores (0.2-10 MDa). The low removal rate of the colloidal size standard observed in Figure 5 can be explained as an inner filter effect of the biofilm matrix. Above a specific molecular weight the available interfacial area of the polymer/particle will be the surface area of the biofilm rather than the area of the internal pore space within the biofilm. The results (Figure 5) support the conclusion that there is an upper limit for biofilm diffusion and that this limit is between 0.5 and 35 MDa.

Conclusion

Dextran degradation in biofilms was investigated to determine the effect of initial molecular weight on the observed removal rates. Variable half order degradation rate expressions showed good correlation to observed bulk phase TOC data. The observed degradation rates were inversely correlated to the molecular weight ($r_A \sim M_W^{-0.2}$) for dextrans in the range from 6 to 500 kDa. The molecular weight of colloidal dextran (>1 MDa) did not fit the correlation for the dissolved polymers, indicating a different degradation regime. Based on estimated diffusion coefficients for different size dextrans it was shown that the molecular weight had no systematic effect on observed reaction rates, but that reduced substrate removal rates could be solely attributed to reduced diffusion coefficients for larger dextrans. It was also concluded that the variable order model provides a simple and robust method to evaluate the effects of substrate M_W on removal rates in biofilms.

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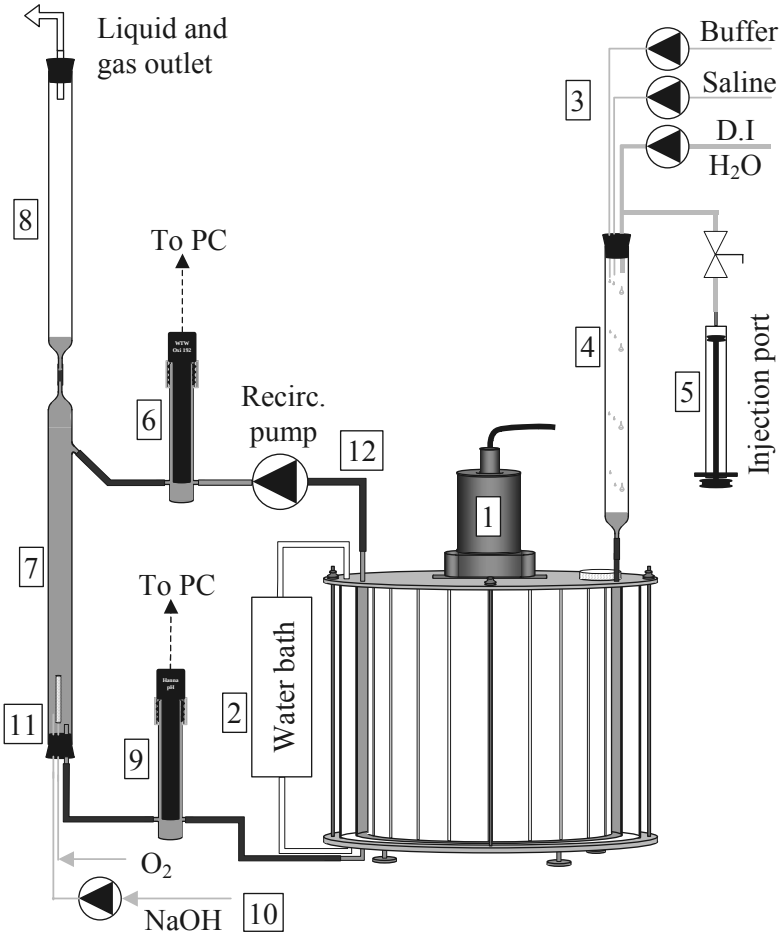

Figure 1. Experimental set up indicating flow directions and reactor connections to external units. 1) Rototorque reactor with water jacket and top drive motor, 2) External water bath with recirculation pump, 3) Inlet pumps, 4) Inlet back-growth preventer, 5) Standard injection syringe, 6) Oxygen probe in flow through cell, 7) Counter current oxygenation column, 8) Oxygenation column debubbler, 9) pH probe in flow through cell, 10) pH control pump, 11) Diffuser for pure oxygen addition.

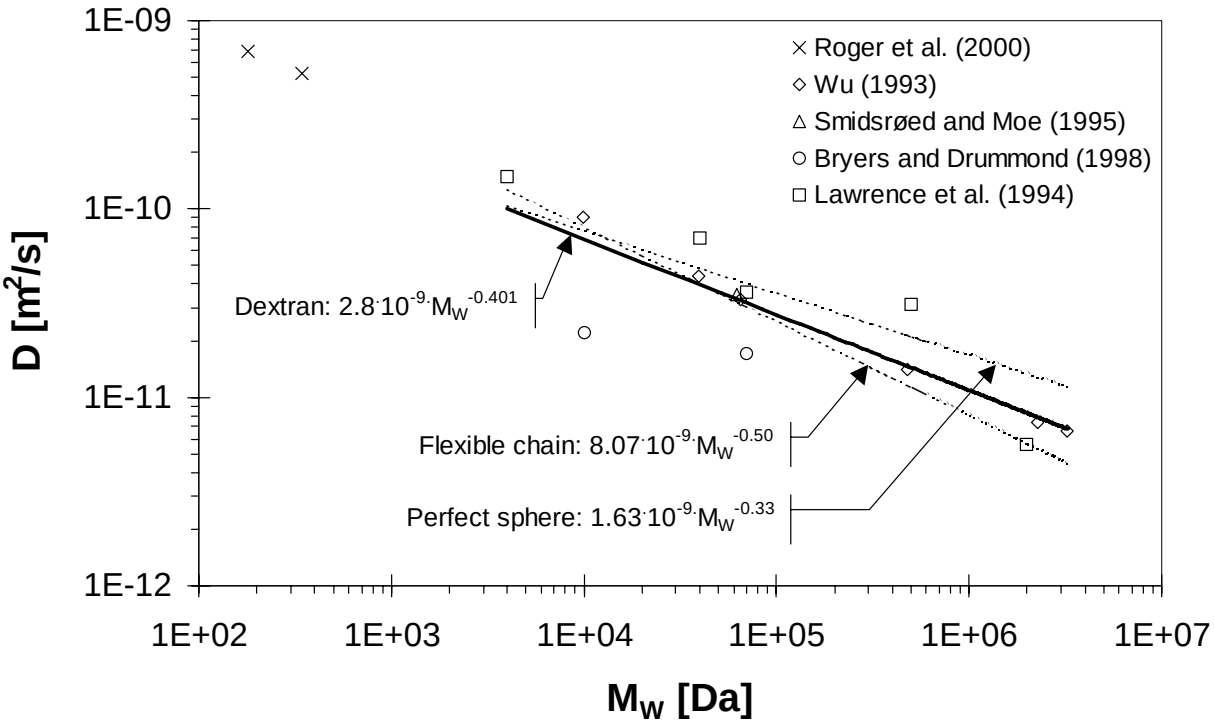

Figure 2. Estimation of dextran diffusion coefficients in water. Models are based on data in the 4-2000 kDa region. Diffusion coefficients for the monomer and dimer (×) are indicated. The solid line shows the best fit using Eq. (8) to the reported dextran data ($R^2 = 0.76$), while the dashed lines show the best fit for perfect spherical and flexible chain conformations.

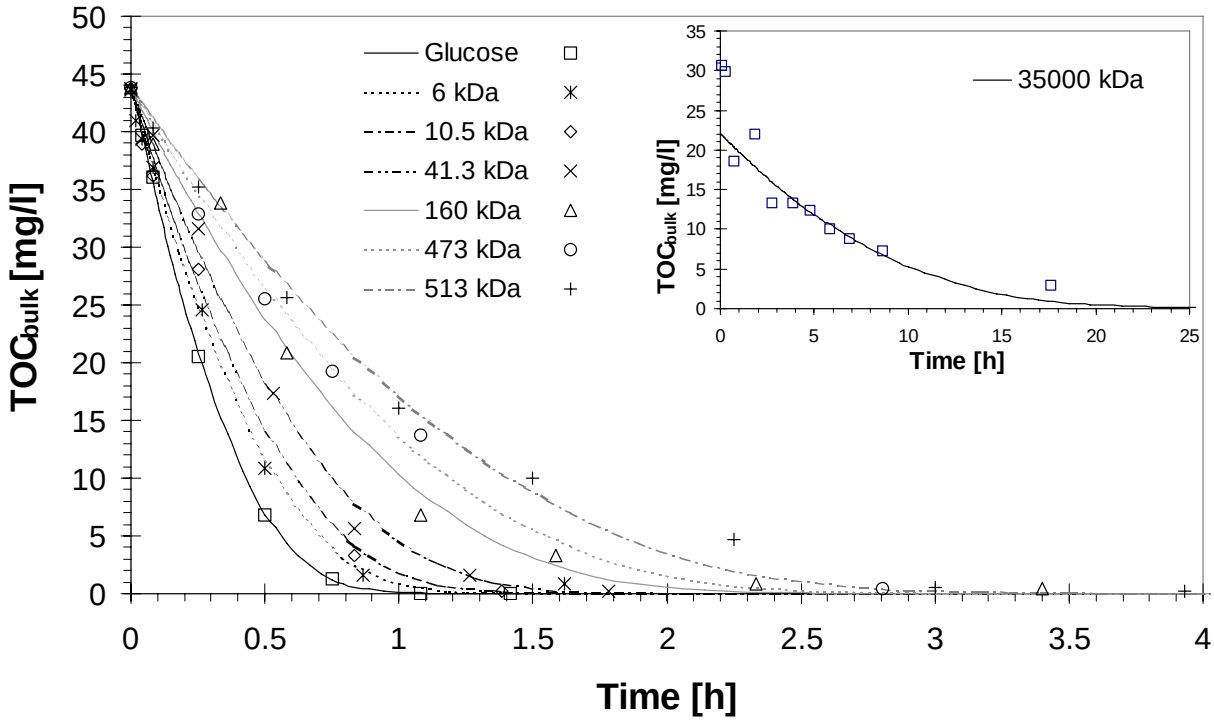

Figure 3. Bulk phase TOC concentrations after injection of 44 mg/l TOC (reactor concentration) dextran standards of varying molecular weight (average molecular weight). Lines show the fitted degradation kinetics using the variable order degradation rate (Eq. 2) and parameters listed in Table 1. Insert show the 36 MDa standard degradation kinetics.

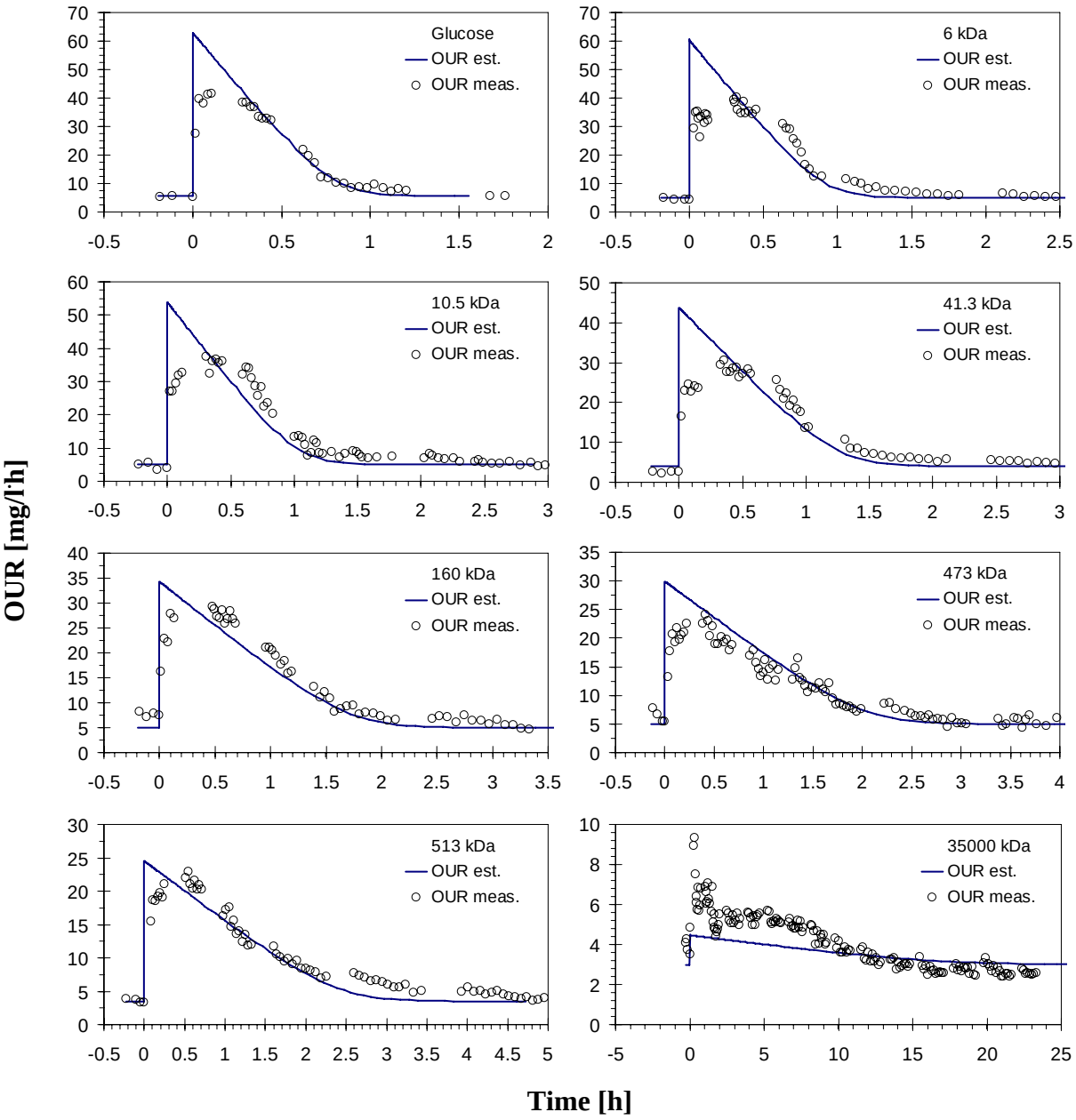

Figure 4. Respiration rates during degradation of dextran size standards. All plots are shown in similar scales in order to compare OUR for the various size standards, except the colloidal standard due to low degradation rates. Lines represent the modelled OUR based on the half order kinetics in Eq. 2, using the estimated half order removal rate coefficient listed for each standard in Table 1.

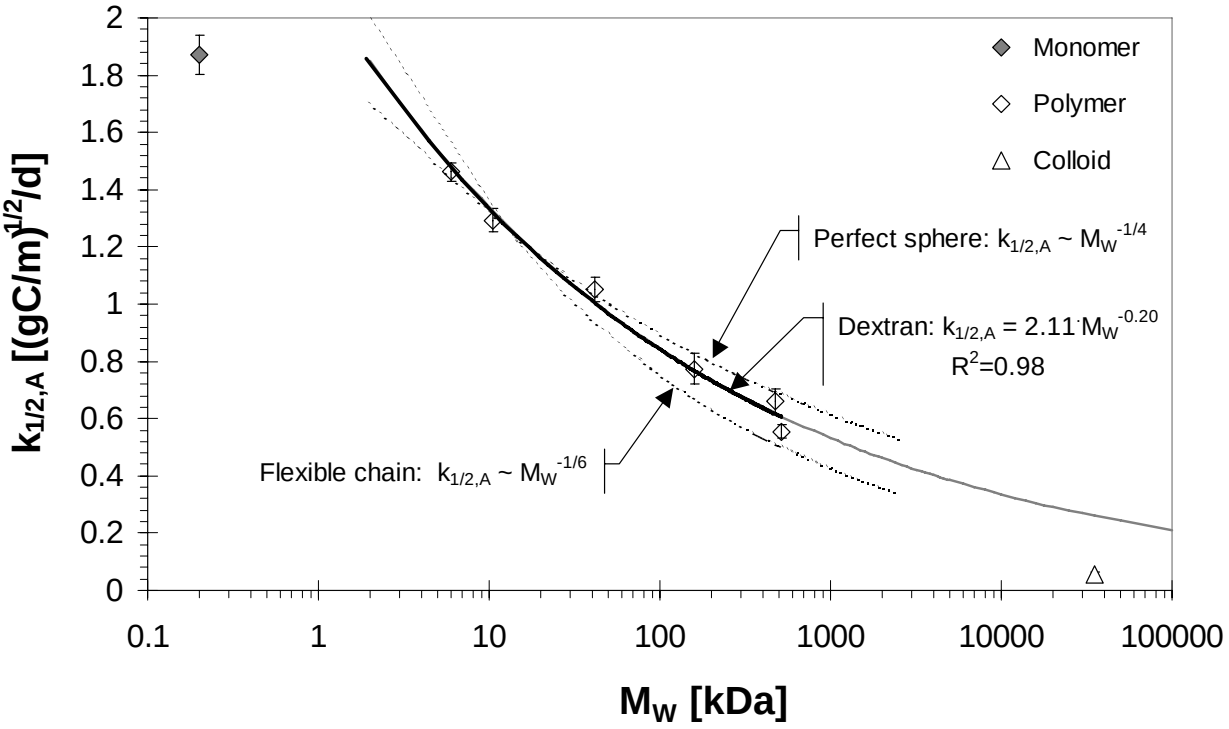

Figure 5. Dependency of variable order hydrolysis rate on the initial molecular weight of dextran. Regression is estimated for the dissolved polymers (solid line). Error bars indicate standard errors. Dashed lines indicate best fit curves assuming spherical and flexible chain configurations. Grey line represents a theoretical half order limited rate as defined by Eq. 2 and 3, using diffusion coefficients from Figure 2 and constant degradation kinetics, $k_{0,f}$. This theoretical line is indistinguishable from the regression line in the range of the experimental data.

Table 1. Effect of dextran initial molecular weight on the calculated half order degradation rate coefficient $k_{1/2,A}$, estimated diffusion coefficients, D_f, and intrinsic degradation rate coefficient, $k_{0,f}$.

Init. Mol. Weight [kDa]	**Est. D_f [(1)]·10^{10} [m^2/s]**	**Est. $k_{0,f}$ [(2)] [kg C/m^3·d]**	**Est. $k_{1/2,A}$ [g C/m^3·d]**	**Std.Error $k_{1/2,A}$ [g C/m^3·d]**	**Number of data points**	**Correlation coefficient $k_{1/2,A}$ [R^2]**
0.18	2.07	98	1.88	±0.07	8	0.9998
6	1.14	362	1.51	±0.03	8	0.998
10.5	0.68	474	1.34	±0.04	7	0.996
41.2	0.39	543	1.08	±0.04	8	0.997
160	0.23	507	0.79	±0.06	8	0.990
473	0.15	566	0.67	±0.05	6	0.996
513	0.14	417	0.57	±0.03	9	0.997
35000	0.026	24	0.057	±0.009	11	0.89

(1) Estimated using the fitted Eq. (8); (2) estimated from OUR using Eq. (9)