Pergamon

Wat. Sci. Tech. Vol. 29, No. 10–11, pp. 479–486, 1994.

0273-1223(94)00328-9
0273–1223/94 $7·00 + 0·00

MODELLING OF EXPERIMENTS WITH COLLOIDAL ORGANIC MATTER IN BIOFILM REACTORS

T. A. Larsen[1] and P. Harremoës

Department of Environmental Engineering, Technical University of Denmark, DK-2800 Lyngby, Denmark

ABSTRACT

A mathematical model for the degradation of colloidal organic matter in biofilm reactors has been developed. Contradictory to existing theories, the model includes bulk liquid hydrolysis as the first important step in the degradation sequence. This leads to unexpected effects of different reactor configurations. The model was successfully verified with native starch as a model substrate. Observed differences in colloid removal capacity between trickling filters and RBC-reactors are well explained by the model.

KEY WORDS

Biofilm, Colloids, Degradation, Enzymes, Extracellular hydrolysis, Modelling, Reactor, Starch.

NOTATION (CARBOHYDRATE MEASURED AS COD).

index 1: Inlet
index 2: Outlet
index V: Volumetric rate
index A: Surface rate

Concentrations [gm^{-3}]:
C_T: Total carbohydrate
S_S: Directly metabolizable substrate
S_A: Analytically defined fraction ≈ S_S
S_L: Large diffusible substrate
S_D: Biofilm-diffusible substrate ($S_D = S_A + S_L$)
X_S: Non-biofilm-diffusible substrate
X_H: Heterotrophic biomass

Concentrations [IUm^{-3}] (IU:internat.units):
S_E: Extracellular enzymes

Reactor configuration:
Q: Flow rate [m^3d^{-1}]
V: Bulk liquid volume [m^3]
A: Biofilm area [m^2]

Biofilm reaction rates:
r_{AM}: Removal rate of C_T [$gm^{-2}d^{-1}$]
p_{AE}: Production rate of enzymes [$IUm^{-2}d^{-1}$]

Bulk liquid reaction rates [$gm^{-3}d^{-1}$]:
r_{VH}: Production rate of S_D
r_{VH}*: Production rate of S_A

Diffusion coefficients: [m^2d^{-1}]:
D_D: Diffusion coefficient of S_D
D_A: Diffusion coefficient of S_A
D_L: Diffusion coefficient of S_L

[1]Present Address: Institut für Hydromechanik und Wasserwirtschaft, ETH-Z, CH-8093 Zürich-Hönggerberg

Rate constants:

μ_{max}: Maximum growth [d^{-1}]
k_{hb}: Bulk liquid hydrolysis (Monod) [$gIU^{-1}d^{-1}$]
k_{hf}: Biofilm hydrolysis (Monod) [$gg^{-1}d^{-1}$]
k_E: Production of enzymes [$IUg^{-1}d^{-1}$]
k_{2Hb}: 2'order bulk liquid hydrolysis [$m^3IU^{-1}d^{-1}$]
k_{0f}: 0'order biofilm degradation [$gm^{-3}d^{-1}$]
$k_{½A}$: Half order removal [$g^{½}m^{-½}d^{-1}$]

Stoichiometric coefficients:

Y: Yield [$g_{COD}g_{COD}^{-1}$]
f_A: r_{VH}^*/r_{VH}

Half saturation constants [gm^{-3}]:

K_X: Bulk liquid hydrolysis of X_S
K_L: Biofilm hydrolysis of S_L
K_A: Biofilm degradation of S_A
K_E: Enzyme production (switching constant)

Dimensionless quantities:

D_H: $\frac{X_{S1} - X_{S2}}{X_{S1}}$ (degree of hydrolysis)

INTRODUCTION

Although nowadays biofilm research is dealing with very complex microbiological phenomena, the understanding of biofilm kinetics is still restricted to the degradation of soluble substrate. Since only a small fraction of the organic matter in wastewater can be considered truly soluble (Levine et al., 1985), this is a severe limitation for our understanding of full scale biofilm reactors for wastewater treatment.

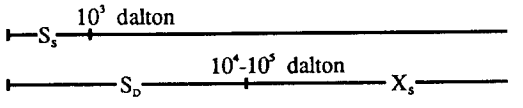
It is widely accepted that soluble substrate is transported into the biofilm by molecular diffusion. Substrate which cannot diffuse into the biofilm is generally assumed to attach to the biofilm surface where it is hydrolyzed to diffusible substrate. On this topic, Bouwer (1987) has presented theoretical work dealing with surface adsorption whereas Stolzenbach (1989) suggested that surface film filtration would be the main mechanism leading to attachment. Bulk liquid hydrolysis (outside of the biofilm) was recently discussed by Sprouse and Rittmann (1990) who conducted an experimental study with a methanogenic biofilm degrading full milk. In their study, no bulk liquid hydrolysis could be detected.

However, in previous work with starch as a model substrate (Larsen and Harremoës, 1994), we found that bulk liquid hydrolysis did take place. Furthermore, we found indications that bulk liquid hydrolysis was a necessary step for transforming non-diffusible substrate into diffusible substrate.

SUBSTRATE FRACTIONS

A fractionation of wastewater organic matter relevant to biofilm treatment was suggested and is here presented in Figure 1. The important distinction is between biofilm **diffusible** and **non-diffusible** substrate (S_D and X_S). In the experiments, however, these fractions could not be distinguished. Only the substrate fraction S_A which is very close to the degradable fraction S_S could be measured (Figure 1b). Since the important variables could not be measured explicitly, the model could only be verified with a combination of experiments and mathematical modelling.

a) Fractions of substrate of theoretical interest:



b) Fractions of substrate measured in this study:

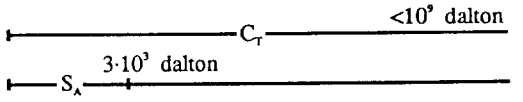


Fig. 1: Fractionation of wastewater organic matter relevant to biofilm treatment.

TABLE 1: SIMPLE MODEL FOR THE BIOFILM DEGRADATION OF COLLOIDAL SUBSTRATE

Component→ Process↓	X_S	S_D	Process rate
Bulk liquid hydrolysis	-1	+1	$k_{2Hb} X_S S_E$
Biofilm substrate removal		-1	k_{0f}
Units	COD	COD	

MODELLING

The concepts of the present experimental study were developed on the basis of the simplified mathematical model presented by Larsen and Harremoës (1994) (Table 1).

In the previous work, an analytical solution to the simplified model was found and the degree of hydrolysis D_H was identified as the central parameter changing the kinetics relative to the well known biofilm kinetics for soluble components:

$$D_H = \frac{X_{S1} - X_{S2}}{X_{S1}} = \frac{1}{1 + \frac{Q^2}{k_{2Hb} \cdot p_{AE} \cdot A \cdot V}} \quad (1)$$

For a deep biofilm (not fully penetrated by diffusible substrate), the surface removal rate of total carbohydrate, r_{AM}, was written:

$$r_{AM} = \frac{k_{1/2A}^2 \cdot A}{2Q} \left[\sqrt{\frac{4Q^2 \cdot C_{T1}}{k_{1/2A}^2 \cdot A^2} \cdot \left(\frac{S_{D1}}{C_{T1}} + D_H \cdot \frac{X_{S1}}{C_{T1}} \right) + 1} - 1 \right] \quad (2)$$

$$k_{½A} = \sqrt{2 D_D k_{0f}}$$

For the design of the experiments, p_{AE} was assumed constant. From the expression for the degree of hydrolysis, it is seen that changes in reactor configuration (V and Q) and C_{T1} are expected to influence the surface removal rate of substrate, r_{AM}. Accordingly, experiments with independent changes in V, C_{T1} and Q were planned. The results predicted by the simple model are presented in Figure 2.

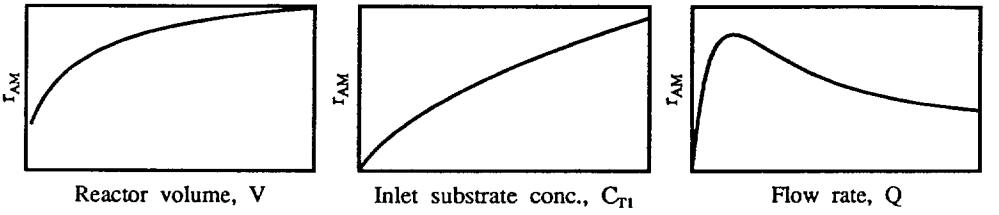


Fig. 2: Surface removal rate of substrate r_{AM} as a function of a) bulk liquid volume V, b) substrate inlet concentration C_{T1} and c) hydraulic flow rate Q.

The assumption that p_{AE} is constant is a specific problem in this description. Often, the production of extracellular hydrolytic enzymes is induced by the end products of the hydrolysis (Priest, 1984). Separate experiments have supported that in the present biofilm system, enzyme production is regulated by an induction mechanism (Keiding, 1991). This means that when more microorganisms are exposed to low molecular weight substrate (deeper penetration of substrate), not only will the observed surface removal rate of substrate increase, but so will the production rate of extracellular enzymes.

In order to simulate the experimental results in detail, the simple model was extended. Enzyme induction and hydrolysis of larger diffusible components S_L to alcohol soluble substrate S_A within the biofilm were included. The extended model is presented in Table 2. Notice, that Monod kinetics has replaced the 0' and 2'order kinetic expressions for hydrolysis and degradation.

TABLE 2: EXTENDED MODEL FOR BIOFILM DEGRADATION OF COLLOIDAL SUBSTRATE.

Component → / Process ↓	X_S	S_A	S_L	S_E	Process rate
Bulk liquid hydrolysis	-1	$+f_A$	$+(1-f_A)$		$k_{hb}\frac{X_S}{K_X+X_S}S_E$
Biofilm hydrolysis		+1	-1		$k_{hf}\frac{S_L}{K_L+S_L}X_H$
Biofilm metabolism		$-\frac{1}{Y}$			$\mu_{max}\frac{S_A}{K_A+S_A}X_H$
Enzyme production				+1	$k_E\frac{S_A}{K_E+S_A}X_H$
Units		COD	COD	IU	

MATERIAL AND METHODS

The experiments were performed at 10°C ±1°C.

Reactor set-up

In Figure 3 the principle of the experimental set-up is shown. The reactor is an annular reactor with a rotating inner drum, originally described by Kornegay and Andrews(1969). The bulk liquid is contained in the gap between the two cylinders and biofilm growth occurs on the outside of the inner rotating cylinder and on the inside of the outer one. Four slides can be removed from the outer cylinder, allowing for measurements of biofilm properties (biofilm thickness etc.). A rotational speed of 200 rpm of the inner cylinder combined with an external recirculation assures that the reactor is ideally mixed and that uniform biofilm growth occurs (Jansen, 1983). Table 3 gives the physical properties of the reactor.

In the recirculation loop, differently sized glass flasks were placed, permitting a variation of the bulk liquid volume. Flasks of approximately one and two liters were used. Vigorous magnetic stirring assured that the flasks were ideally mixed. Simulated tracer curves have shown that with the recirculation rate applied, the entire system could be considered as practically ideally mixed. It was assumedthat biofilm growth only took place in the biofilm reactor and not on the walls of the inserted flasks.

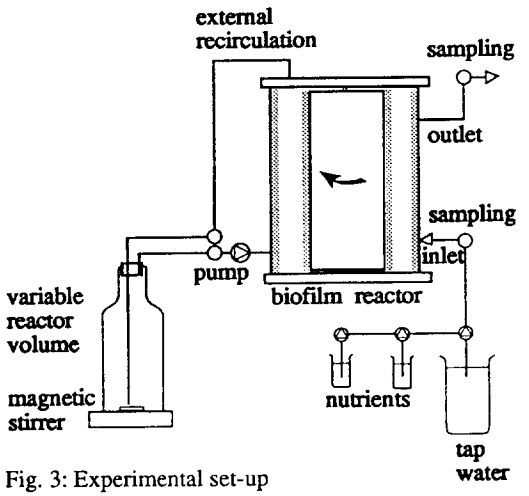


Fig. 3: Experimental set-up

TABLE 3: PHYSICAL PROPERTIES OF THE BIOFILM REACTOR

Volume	$9.54 \cdot 10^{-4}$ m^3
Area stator	0.089 m^2
Area rotor	0.070 m^2
Height of reactor	0.250 m
Gap between cylinders	0.012 m
External recirculation	1.4 m^{3}d^{-1}

Model substrate

Experiments were performed with native potato starch (Merck 1259). Native potato starch consists of 25 % amylose, a linear polymer of glucose (average molecular weight $5 \cdot 10^5$ dalton) and 75 % amylopectin, a branched polymer of glucose (average molecular weight $3 \cdot 10^8$ dalton) (Swinkels, 1985). Magnetic stirring of the substrate fed to the reactor was necessary in order to prevent sedimentation of starch.

Feed composition of inorganic nutrients

Macro nutrients [gm^{-3}]: KH_2PO_4: 45.4; $NaH_2PO_4 \cdot H_20$: 27.6; $(NH_4)_2SO_4$: 17.6; NH_4Cl: 164.0; $CaCl_2$: 14.8; $MgCl_2 \cdot 6H_20$: 27.1. Micro nutrients [10^{-3} gm^{-3}]: $MnCl_2 \cdot 4H_2O$: 527; $CuCl_2 \cdot 2H_2O$: 228; $CoCl_2 \cdot 6H_2O$: 317; $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$: 231; $Na_2B_4O_7 \cdot 10H_2O$: 127; $ZnCl_2$: 363; $FeSO_4 \cdot 7H_2O$: 3700.

Yeast extract (Difco Bacto 0127-02) was fed at inlet concentrations of 2 gm^{-3}.

Measurement of biofilm thickness

Biofilm thickness was measured by a microscopic technique described by Siegrist and Gujer (1985).

Sampling

200 ml samples were taken from the in- and outlet of the reactor and thoroughly mixed. Carbohydrate analyses were performed directly (without previous filtration) although polymeric substances from detached biomass may interfere with the analysis. Filtration, however, removes part of the substrate and was therefore not possible.

Pre-treatment of glassware

Additional bulk liquid volume. Glass flasks inserted in the recirculation loop in order to create additional bulk liquid volume were rinsed once a day in concentrated HCl. During experiments, new flasks were used. Since the inner wall of a new flask is not covered with polymeric substances, it is only slowly colonized by microorganisms. For the experiment described in this paper, two new flasks (one and two liter) were used for a period of two days. A flask stayed in the system only for a few hours and was then rinsed in concentrated HCl before being reused. Due to this procedure, no traces of biofilm growth were detected in the flasks.

Glassware for analytical purposes. Traces of carbohydrate were removed from all glassware used for carbohydrate separation and analysis. The glassware was soaked in 0.1 M HCl for at least 12 hours. After rinsing, it was burned in a 250°C oven for 4 hours.

Analytical methods

Carbohydrate analysis. Total carbohydrate was measured according to a spectrophotometric method described by Dubois et al. (1956). Measurements were performed at 489 nm and standardized against glucose. Inorganic nutrients did not affect the results of the analysis.

Protein analysis. Assuming the protein content of the biofilm to be representative for the active biomass in the biofilm, a protein analysis was used for measuring biomass density. The protein content was assumed to be 50 % of the biomass dry matter. For the analysis, a modified method of Bradford (1976) as described by

Gälli and McCarty (1989) was used. Biofilm protein content was standardized against Bovine Serum Albumin and measurements were performed in the range of 0-120 gm^{-3}.

Separation of substrate fractions. Starch and the low molecular weight fraction of oligosaccharides (S_A) were separated by precipitating starch following the addition of ethanol. S_A could then be measured in the supernatant. The precipitation procedure was described earlier (Larsen and Harremoës, 1994).

EXPERIMENTAL RESULTS

Observed surface removal rates of substrate, r_{AM}, presented in Figure 4 were in accordance with the predictions of the simple mathematical model presented in Figure 2. Traditional biofilm kinetics for soluble components cannot explain the decrease of surface removal rates observed at higher flow rates and lower reactor volume.

For simulating the results according to the extended model presented in Table 2, the interactive computer program for biofilm simulation BIOSIM (Reichert et al., 1989) was used. The interesting results from the experiment are the surface removal rate of substrate (r_{AM}), the bulk liquid production of S_A (r_{VH}*) and the resulting bulk liquid concentration of low molecular weight components (S_A). The model constants are presented in Table 4 and the simulation results are compared to the experimental results in Figure 4 and Figure 5.

TABLE 4: MEASURED, ESTIMATED AND CALIBRATION PARAMETERS OF THE MODEL

Symbol	Unit	Value	Comments
Measured parameters			
X_H	$g\ m^{-3}$	30000	Biomass COD = 2 x biomass protein
μ_{max}/Y	$g\ g^{-1}d^{-1}$	6.7	Determined in a separate experiment with substrate in excess
k_{hf}	$g\ g^{-1}\ d^{-1}$	8.0	Determined in a separate experiment with substrate in excess
k_E	$IU\ g^{-1}d^{-1}$	0.11	As reported by Keiding (1991)
Estimated parameters			
Y	$g\ g^{-1}$	0.6	
S_{D1}/C_{T1}	[-]	0.25	Amylose assumed diffusible
K_A,K_L,K_X	$g\ m^{-3}$	1	
K_E	$g\ m^{-3}$	$1 \cdot 10^{-2}$	Mathematical switching constant
D_A	$m^2\ d^{-1}$	$4.4 \cdot 10^{-5}$	Diffusion coefficient for S_D (approximate value from size)
D_L	$m^2\ d^{-1}$	$1.6 \cdot 10^{-5}$	Diffusion coefficient for S_L (approximate value from size)
Model calibration			
k_{hb}	$g\ IU^{-1}\ d^{-1}$	$5.5 \cdot 10^{-2}$	
f_A	[-]	0.27	

DISCUSSION

The comparison of experimental data with simulated results presented supports that bulk liquid hydrolysis is responsible for transforming non-diffusible colloidal substrate X_S into diffusible substrate S_D. The simple model (Table 1) is able to predict qualitatively all changes in surface removal rate of substrate, r_{AM}, obtained with changes in reactor configuration. With the extended model (Table 2) , all experimental data (surface removal rate of substrate, r_{AM}, bulk liquid production of S_A, r_{VH}*, and the resulting bulk liquid concentration of low molecular weight components, S_A) are well predicted.

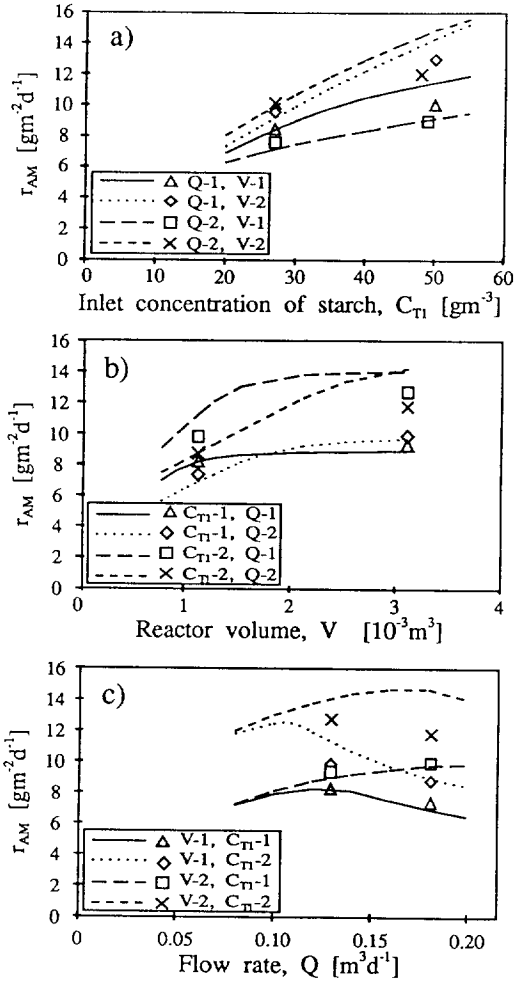


Fig. 4: Surface removal rate (r_{AM}) as a function of different parameters. Lines indicate model predictions (BIOSIM) for reactor configurations identical to or close to experimental conditions.

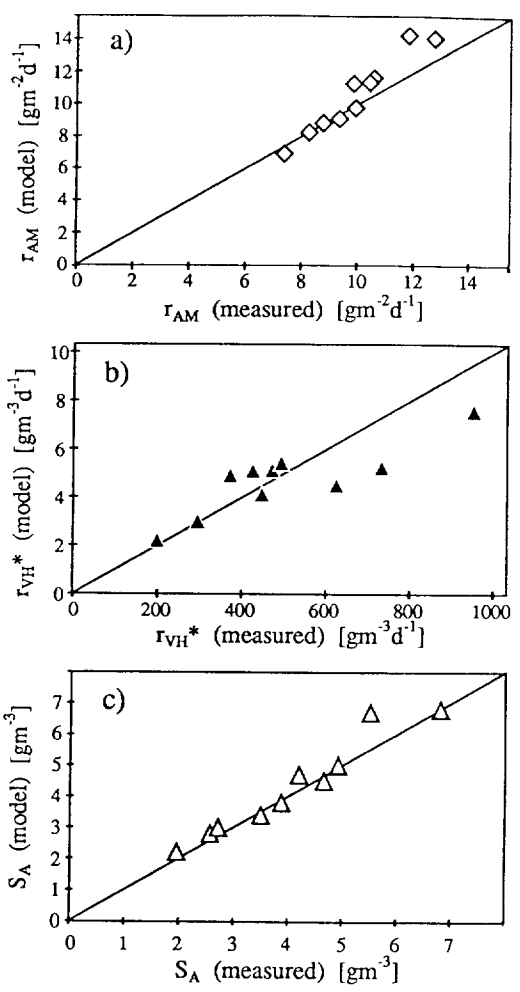


Fig. 5: Values from BIOSIM modelling versus experimental results. Straight lines represent perfect agreement between model and experiment.

V-1 = 0.0011 m^3, V-2 = 0.0031 m^3
Q-1 = 0.13 m^{3}d^{-1}, Q-2 = 0.18 m^{3}d^{-1}
C_{T1}-1 = 27 gm^{-3}, C_{T1}-2 = 50 gm^{-3}

a) Surface removal rate (r_{AM})
b) Bulk liquid production of S_A (r_{VH}*)
c) Resulting bulk liquid concentration of S_A (S_{A2}).

The practical importance of the models depends on the amount of organic matter in wastewater needing hydrolysis before being degraded in biofilm reactors. Bulk liquid hydrolysis as an important 'pre-treatment' of colloidal substrate may explain full scale experience with poor removal capacity of colloidal matter in trickling filters (Parker et al., 1990) as compared to RBC reactors which contain larger bulk liquid volumes. According to the simple model, the degree of hydrolysis, and thereby the availability of substrate, increases with a decreasing combined hydraulic loading rate, $Q^2/(AV)$. Since the typical combined hydraulic loading rate is 2.6 md^{-2} for a trickling filter and only 0.4 md^{-2} for an RBC-reactor (Larsen and Harremoës, 1994), our model can explain the difference of performance between the two types of treatment plants.

CONCLUSIONS

1. In the present system, non-diffusible organic matter was transformed into diffusible substrate by bulk liquid hydrolysis. Only diffusible substrate was available for biofilm degradation.

2. The combined hydraulic loading rate ($Q^2/(AV)$) is the important design parameter for the degradation of non-diffusible substrate. For increasing combined loading rate, the degree of hydrolysis decreases. This results in a decrease of the surface removal rate of substrate.

3. A numeric model has been formulated which includes bulk liquid hydrolysis, hydrolysis and degradation within the biofilm and an induction mechanism for enzyme production. With this model all experimental results were well simulated.

4. The practical relevance of the model depends on the amount of non-diffusible colloids in wastewater which are transformed into diffusible substrate by bulk liquid hydrolysis. Full scale experience with poor removal capacity of colloids in trickling filters support that the mechanism is important for treatment of wastewater in biofilm reactors.

REFERENCES

Bouwer, E.J. (1987) Theoretical Investigations of Particle Deposition in Biofilm Systems. *Wat. Res.*, **21**, 1489-1498.

Bradford, M.M. (1976) A Rapid and Sensitive Method for the Quantification of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. *Analytical Biochemistry*, **72**, 248-254.

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A. and Smith, F. (1956) Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*, **28**, 350-356.

Gälli, R. and McCarty, P.L. (1989) Biotransformation of 1,1,1-Trichlorethane, Trichlormethane, and Tetrachlormethane by a Clostridium sp. *Applied Environmental Microbiology*, **55**, 837-844.

Jansen, J. la Cour (1983) Fixed Film Kinetics - Removal of Soluble Substrates in Fixed Films. Ph.D.Thesis. Department of Environmental Engineering, Technical University of Denmark.

Keiding, K. (1991) Mechanism and Kinetics for Decomposition of Polymere Compounds in a Biofilm. Non-published report from the Department of Environmental Engineering, Technical University of Denmark.

Kornegay, B.H. and Andrews, J.F. (1969) Characteristics and kinetics of biological fixed film reactors. Department of Environmental System Engineering. Clemson University, South Carolina, USA.

Larsen, T.A. and Harremoës, P. (1994) Degradation Mechanisms of Colloidal Organic Matter in Biofilm Reactors. *Wat. Res.* **28** 1443-1452.

Levine, A.D., Tchobanoglous, G. and Asano, T. (1985) Characterization of the Size Distribution of Contaminants in Wastewater: Treatment and Reuse implications. *Journal of Water Pollution Control Federation*, **57**, 805-816.

Parker, D. S., Lutz, M. P. and Pratt, A. M. (1990) New Trickling Filter Applications in the U.S. *Wat. Sci.Tech.* **22** (1/2), 215-226.

Priest, F.G. (1984) *Extracellular Enzymes.* Van Nostrand Reinhold. Workingham. Berkshire.

Reichert, P., Ruchti, J. and Wanner, O. (1989) BIOSIM. Interactive Program for the Simulation of the Dynamics of Mixed Culture Biofilm Systems on a Personal Computer. Users Guide. EAWAG, Switzerland.

Siegrist, H. and Gujer, W. (1985) Mass Transfer Mechanisms in a Heterotrophic Biofilm. *Wat. Res.*, **19**, 1369-1378.

Sprouse, G. and Rittmann, B.E. (1990) Colloid Removal in Fluidized-Bed Biofilm Reactor. *Journal of Environmental Engineering*, **116**, 314-329.

Stolzenbach, K.D. (1989) Particle Transport and Attachment. In Characklis, W.G. and Wilderer, P.A. (eds.): *Structure and Function of Biofilms,* 33-47. Dahlem Konferenzen, 1988. John Wiley and Sons.

Swinkels, J.J.M. (1985) Composition and Properties of Commercial Native Starches. *Starch/Stärke*, **37**, 1-5.

Acknowledgements: The work described in this paper was entirely supported by a grant from the Danish Environmental Biotechnology Program.