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# REMOVAL OF SOLUBLE SUBSTRATES IN FIXED FILMS

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ABSTRACT

Experimental results with removal of soluble substrates in fixed films are presented. Experiments are performed with denitrification and with oxidation of methanol, acetic acid and glucose in a laboratory fixed film reactor.

The experiments confirm the predictions of the half order reaction model, taking diffusion of the substrates and zero order intrinsic reaction rate of the bacteria as the dominating processes in the film. The experiments enable calculation of the kinetic parameters: diffusion coefficients, intrinsic reaction rates and half order rate constants for nitrate, methanol, acetic acid, glucose and oxygen within fixed films.

KEYWORDS

Kinetics; fixed films; denitrification; oxidation of organics.

## INTRODUCTION

Fixed film reactors have been reconsidered for wastewater treatment during the last twenty years. Plastic media and new process configurations such as rotating disks and fluidized filters have appeared. Furthermore the increasing need for treatment of new types of wastewater has increased the interest in fixed film reactors for treatment of both domestic and industrial wastewater. The traditional approach where the treatment plant has been considered more or less as a "black box" does not suffice. Deeper insight into all processes and phenomena related to the fixed film reactors has to be established.

Many different types of phenomena have to be considered in order to establish a sound basis for design and operation of fixed film reactors. The hydraulics, that is the macro-transportation of water and substrates within the reactors, differ very much from one reactor type to another. Transportation of gasses which take part in the biological processes also differs from system to system, just as the significance of a possible liquid film layer. However, common to all types of reactors are the processes within the fixed film. The substrates may be brought to the surface of the bacterial layer in many different ways, but inside the biofilm the substrates

have to be transported, in soluble form, to the bacteria where the reaction takes place, and the reaction products have to be transported out again. A conceptual model corresponding to this description of the fundamental processes in fixed film is illustrated in Fig. 1. The concepts are in close agreement with those stated by others, including Atkinson and Fowler, 1974; Harremoës and Riemer, 1975; Harremoës, 1978; La Motta, 1974; Williamson and McCarty 1976a, 1976b. In all cases the transportation of the substrates, and reaction products has been taken as Fick'ian diffusion, whereas several proposals for the reaction rate inside the biofilm have been given.

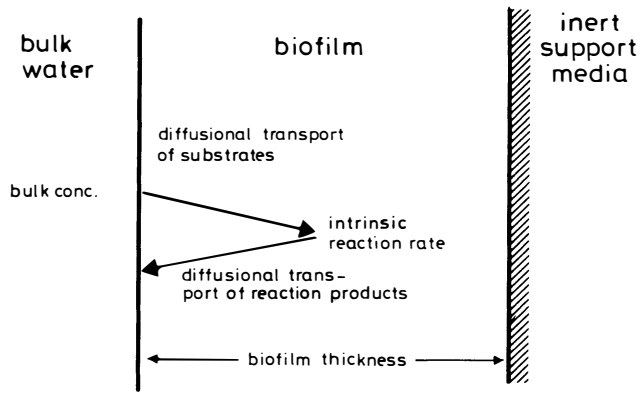


Fig. 1 Conceptual model for removal of soluble substrates focusing on phenomena inside the film.

The intrinsic process is assumed to follow Monod-type kinetics, but the Monod constant is low in many cases, which means that the intrinsic reaction rate can be taken as zero order for most practical applications, as stated by Harremoës, (1978A).

This simplicity of the mathematics leads to a very simple description of the bulk processes. As derived by Harremoës, (1976, 1978), or by Harremoës and Riemer (1975), the diffusion leads to a bulk process which is either half or zero order with respect to the bulk concentration of the substrate under consideration. The following equations summarize the results.

Zero order bulk reaction

$$r_a = k_{0a} = k_{0f} L \qquad \text{valid for} \quad \beta = \sqrt{\frac{2D\, C^*}{k_{0f} L^2}} \geq 1 \qquad (1)$$

Half order bulk reaction

$$r_a = k_{\frac{1}{2}a} C^{*\frac{1}{2}} = \sqrt{2D\, k_{0f}}\, C^{*\frac{1}{2}} \qquad \text{valid for } \beta < 1 \qquad (2)$$

where

$r_a$ is the removal rate per unit area biofilm surface ($g\ m^{-2} s^{-1}$)

$k_{0a}$ is the zero order removal rate per unit area ($g\ m^{-2} s^{-1}$)

$k_{\frac{1}{2}a}$ is the half order rate constant per unit area ($g^{\frac{1}{2}} m^{-\frac{1}{2}} s^{-1}$)

$k_{0f}$ is the intrinsic zero order removal rate in the biofilm ($g\ m^{-3} s^{-1}$)

L is the thickness of the biofilm (m)

D is the coefficient of molecular diffusion in the biomass ($m^2 s^{-1}$)

$C^*$ is the bulk concentration at the surface of the biofilm ($g\ m^{-3}$)

$\beta$ is a dimensionless constant called the "penetration ratio"

The equations state that the bulk process becomes zero order, independent of the substrate concentration, when the substrate penetrates the biofilm fully ($\beta \geq 1$). At lower bulk concentrations, the diffusion limitation leads to partial penetration of the biofilm and a bulk reaction dependent of the bulk substrate concentration to the half power becomes the result.

Biological processes are redox processes where either electron donor or acceptor is the rate limiting substrate. In case of diffusion limitation equation (2) has to be supplemented with a calculation to show which one of the substrates is rate limiting and determines the overall reaction rate.

The component (donor or acceptor) that penetrates the least is rate limiting, leading to the following condition for change of limiting substrate:

$$\frac{C_d^*}{C_a^*} = \frac{D_a}{D_d} \frac{k_{0fd}}{k_{0fa}} = \frac{D_a}{D_d} M \qquad (3)$$

where

$C_d^*$ and $C_a^*$ are the bulk concentrations of the donor and acceptor ($g\ m^{-3}$)

$D_d$ and $D_a$ are the corresponding diffusion coefficients ($m^2\ s^{-1}$)

$k_{0fd}$ and $k_{0fa}$ are the corresponding zero order intrinsic reaction rates ($g\ m^{-3}\ s^{-1}$)

L is the biofilm thickness (m)

M is the stoichiometric consumption ratio ($g\ g^{-1}$)

Harremoës and Riemer (1975), Riemer (1977), and Riemer and Harremoës (1978) describe experiments in a denitrifying down-flow filter confirming equation (1, 2, and 3). The purpose of the experiments described in the following is to demonstrate the validity in general of these equations for removal of soluble substrates in fixed film under conditions as well defined as possible.

## EXPERIMENTAL EQUIPMENT AND PROCEDURES

An experimental reactor suited for examination of the process inside fixed films was developed (details of the reactor construction and performance are given in Kristensen and Jansen (1980)). The experimental set-up for experiments with denitrification and with oxidation of organics is shown in Fig. 2.

The reactor is the result of a development based on the principles described by Kornegay and Andrews (1969) and La Motta (1974).

The reactor volume of about one litre is situated between two cylinders of which the inner is rotating. The rotation ensures a totally mixed bulk phase and creates a hydraulic shear resulting in an even distribution of the biofilm growing on the walls of the cylinder. Four dovetailed slits are placed as integrated part of the outer, stationary cylinder wall. They can be taken up and part of the film can be taken out to be used for measurements of the thickness. Details of the measuring technique can be found in Kristensen and Jansen (1980) or in Kristensen and Christensen (1982).

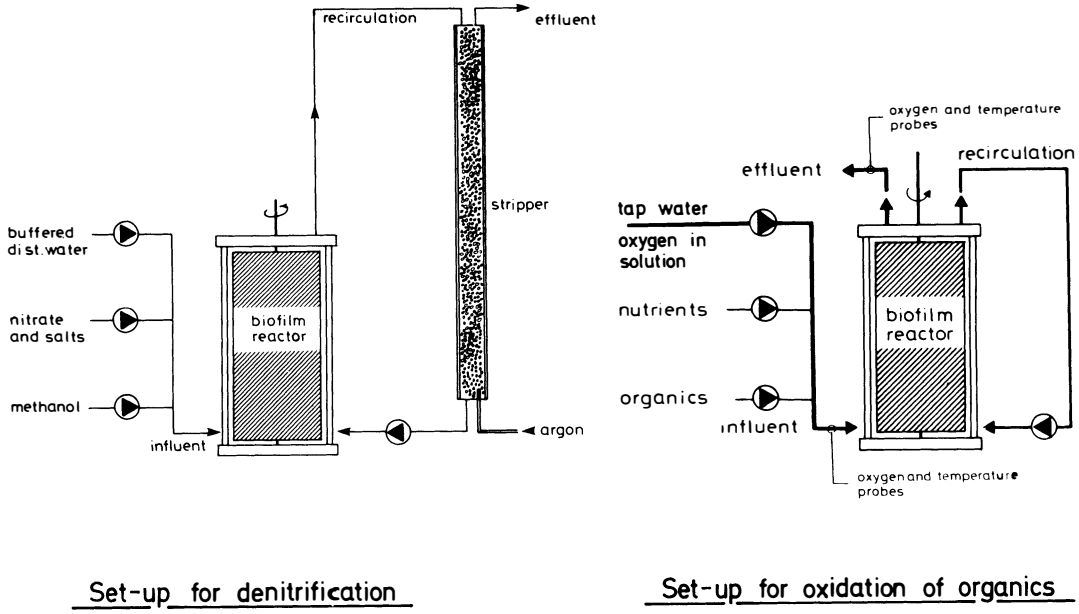


Fig. 2 Experimental set-up for examination of kinetics of soluble substrates in fixed films.

The reactor has been used for experiments with denitrification and with oxidation of organics in a slightly different set-up as seen in Fig. 2 . During denitrification, nitrogen gas is produced, leading to bubble formation at the rear of the biofilm, unless the bulk concentration of molecular nitrogen is kept low. The stripping device shown in Fig. 2 serves that purpose. The significance of nitrogen bubble formation in fixed films and the practical consequence for denitrification in fixed film reactors are described by Harremoës, Jansen and Kristensen (1980). In both cases the reactor is provided with an external recirculation serving two purposes. Total mixing of the bulk water is ensured, independently of the inflow rate. Formation of Taylor-currents (see Coles, 1965; Kristensen and Jansen, 1980; Taylor, 1923) is effectively suppressed. Such currents would disturb the homogeneous growth of the biofilm.

The experimental arrangements enable easy verification of the kinetic model and determination of the kinetic parameters: the diffusion coefficient, the intrinsic removal rate, and the half order rate constant. Considering one substrate only (all other substrates in excess), experiments at high concentrations, where equation (1) is valid, enable determination of the intrinsic removal rate, $k_{0f}$ when the surface removal rate $r_a$ and the biofilm thickness L are measured. At low concentrations where equation (2) is valid, the half order rate constant, $k_{\frac{1}{2}a}$, is found when $r_a$ and bulk concentration $C^*$ are known. Note that the bulk concentration equals the outlet concentration since the bulk of the water is totally mixed. Fig. 3 shows a plot of the surface removal rate of such an ideal, fictitious experiment with planned experimental results indicated, together with the prediction of the kinetic model.

Experiments with examination of change of limiting substrates (equation 3) are performed simply as an extension of the experiments with one component shown in Fig. 3. Figure 4 shows the principle. As a supplement to the one-component experiments, experiments are performed such that the level of the former substrate in excess is reduced until a level where it becomes potential limiting. The actual limitation is then

found experimentally. Accordingly, the validity of equation 3 can be tested.

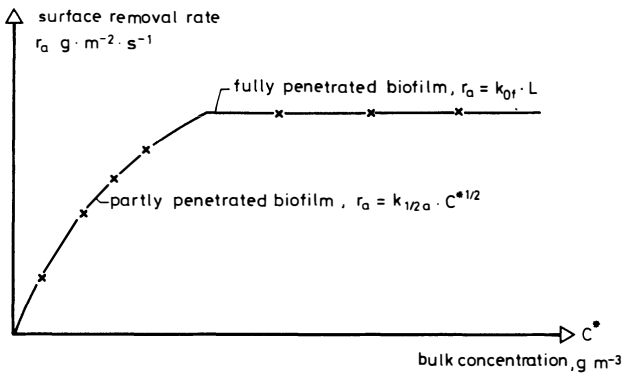


Fig. 3 Plot of an ideal, fictitious one-component experiment. Planned experimental results are indicated.

The change of limiting substrate can be illustrated directly in a dimensionless plot of all experimental data, as shown in Fig. 5, covering situations of rate limitation by either of the two substrates.

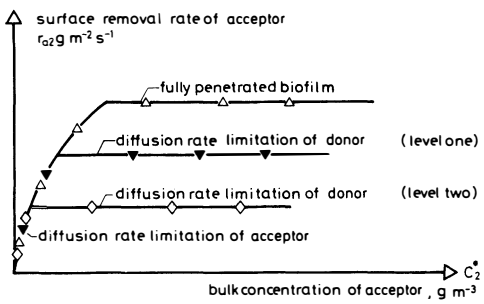


Fig. 4 Plot of an ideal fictitious two-component experiment. Planned experimental results are indicated.

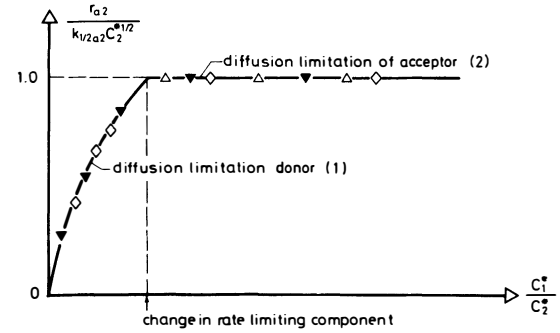


Fig. 5 Dimensionless plot of an ideal two-component experiment demonstrating change of limiting component directly.

## KINETIC EXPERIMENTS

Kinetic experiments with denitrification and with oxidation of different organics have been performed. The fixed film has been grown without substrate depletion prior to the experiments. Exponential increase of the surface removal rate has been found in all cases. The film has been plane and well suited for experiments with substrate removal kinetics as described in the former section. Details of the experiments can be found in Jansen (1983).

Nitrogen Removal

Figure 6 shows results from three experiments with removal of nitrate in fixed films. The experiments are performed with a biofilm at three different thicknesses.

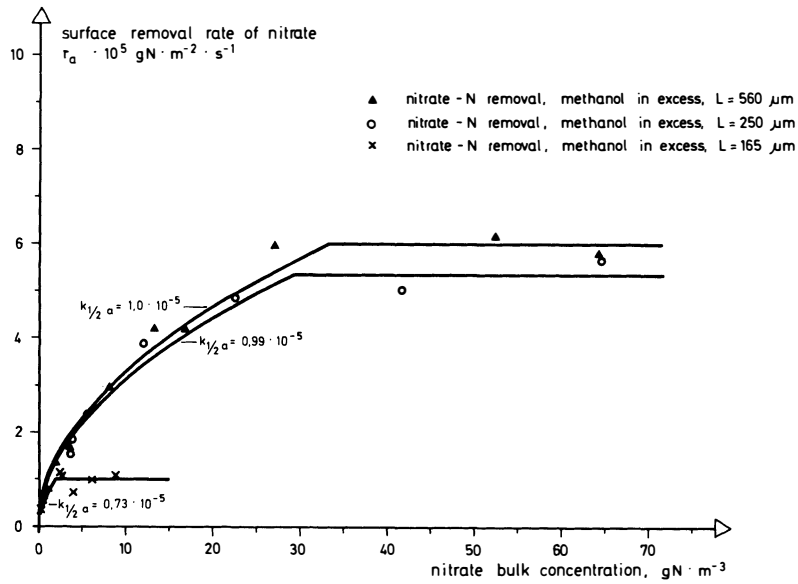


Fig. 6 Experimental results from three experiments with nitrate. The experiments are performed at biofilm thicknesses of 165, 250 and 560 µm.

The experimental points are supplemented by the best fit to the data assuming that the kinetic model is valid. It is seen that the predicted parabolic dependence of surface removal rate to bulk substrate concentration at low concentration is valid, and that the surface removal rate becomes constant at high concentrations. The half order rate constant changes a little as the biofilm thickness increases, illustrating that growth of a biofilm may change the kinetic properties of the film.

Oxygen Consumption

Figure 7 shows results from three experiments with oxygen removal in fixed films grown aerobically on methanol, acetid acid and glucose, respectively. In all cases the proposed dependence of the surface removal rate on the bulk substrate concentration is demonstrated.

Removal of Organics

Figure 8 shows results from three experiments with removal of methanol, acetic acid and glucose, respectively, in aerobic films. As for removal of nitrate and oxygen the proposed dependence exists. For glucose the bulk concentration has been too low to penetrate the biofilm fully.

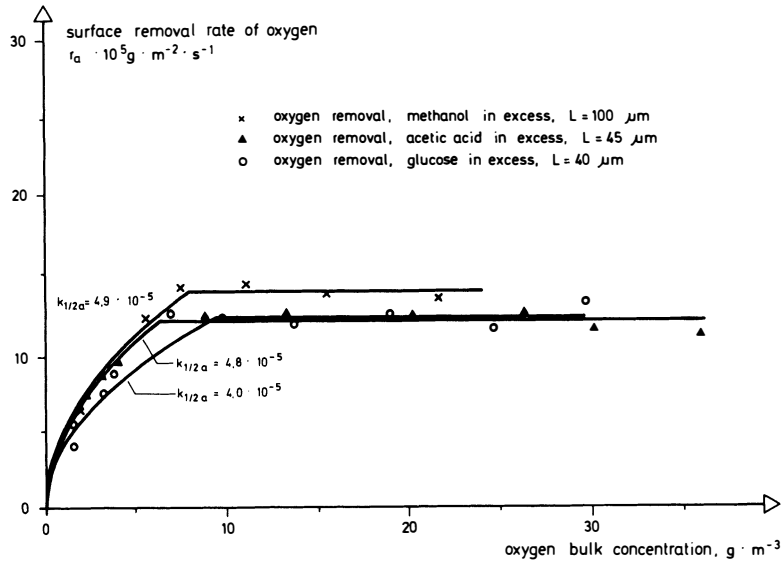


Fig. 7 Experimental results from three experiments with oxidation of organics. Methanol, acetic acid and glucose have been in excess during one experiment each.

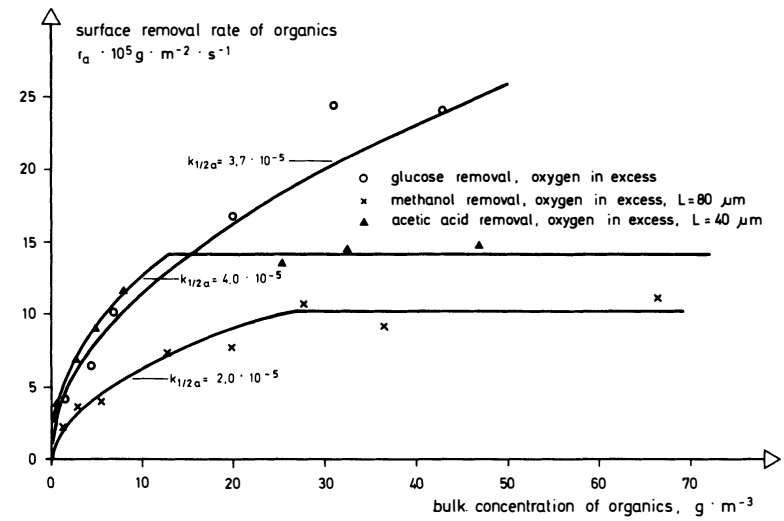


Fig. 8 Experimental results from three experiments with oxidation of methanol, acetic acid and glucose. Oxygen has been in excess in all experiments.

Methanol has been used for experiments with denitrification and oxidation of organics. Fig. 9 shows results with methanol removal in both kinds of films.

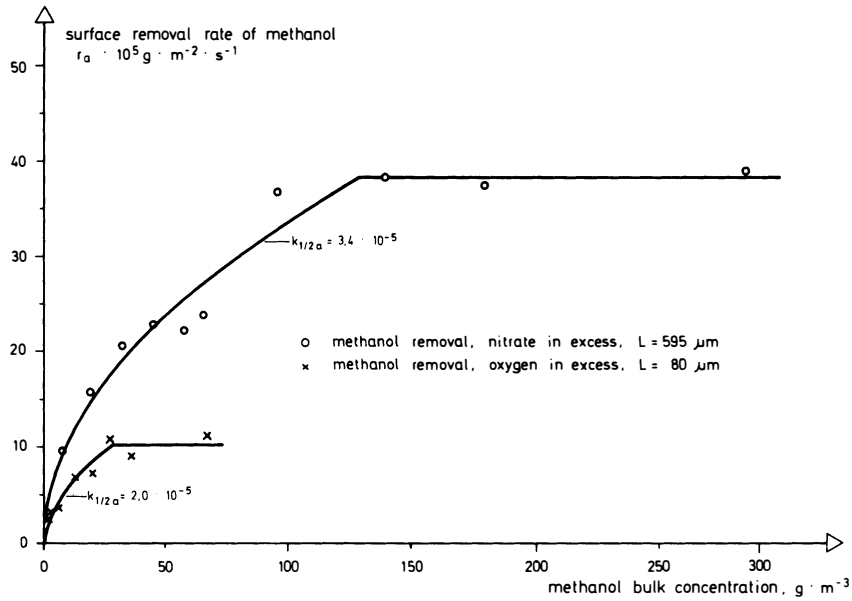


Fig. 9 Experimental results from two experiments with methanol removal. Nitrate and oxygen have been electron acceptor in one experiment each.

## Change of Limiting Substrate

**Figure 10 shows the results from an extended experiment with oxidation of acetic acid.**

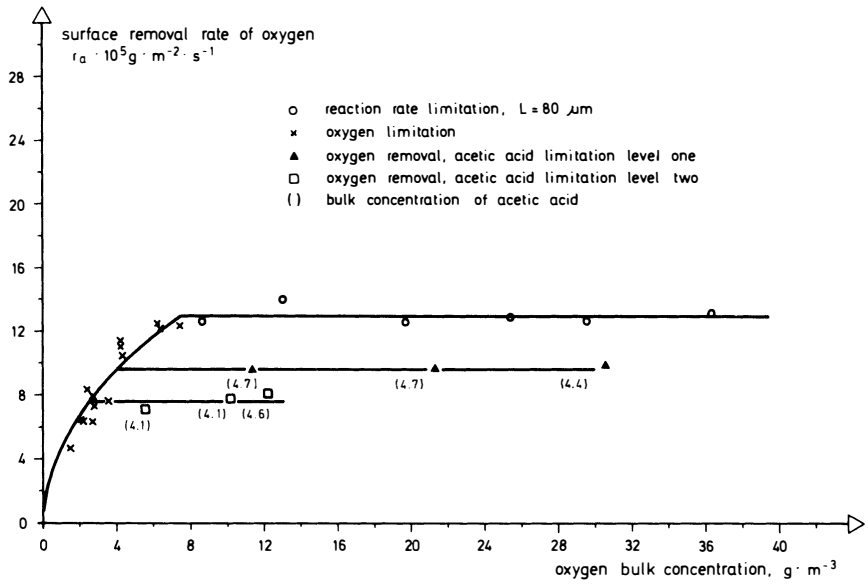


Fig. 10 Experimental results from an experiment with oxidation of acetic acid, demonstrating reaction rate limitation and diffusion limitation to oxygen and acetic acid removal.

The figure shows the oxygen removal rate, but the rates of acetic acid can be found from the stoichiometry of the process. The figure demonstrates the change of rate limitation from reaction rate limitation (no substrate limiting) to oxygen limitation. Furthermore two levels with acetic acid limitation are shown. The direct illustration of change of limiting substrate is shown in Fig. 11. Data from Fig. 10, where one of the substrates is limiting, are combined with further experimental data, where acetic acid is limiting. The change of limiting substrate is seen to take place at a proportion between bulk acetic acid and oxygen concentrations of 1.4 g acetic acid/g oxygen. Figure 12 shows the dimensionless plot of results from a kinetic experiment with denitrification similar to Fig. 11. The change of limiting substrate is seen to take place at a concentration ratio of 2 g methanol/g nitrate-N.

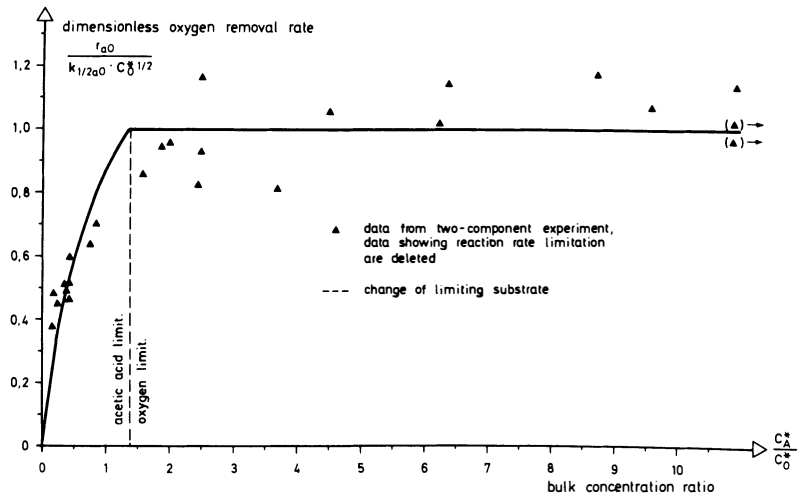


Fig. 11 Dimensionless illustration of change in rate limiting component for oxidation of acetic acid in a biofilm.

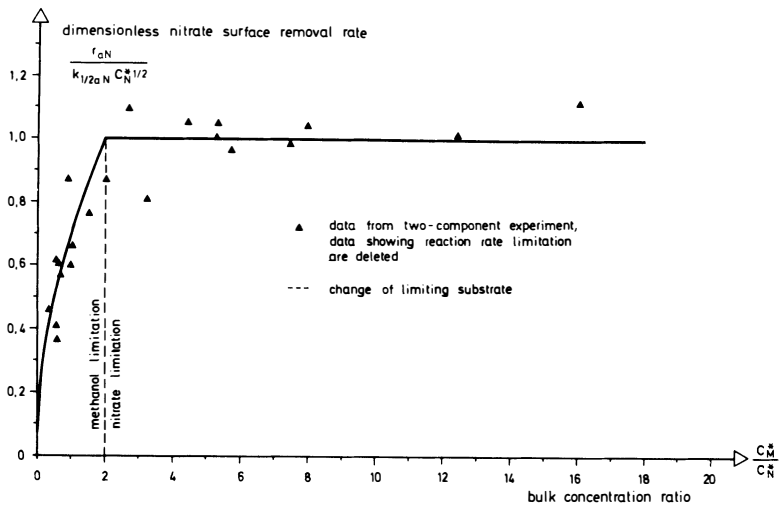


Fig. 12 Dimensionless illustration of change in rate limiting component for a denitrifying biofilm.

## DISCUSSION

Figures 6 to 12 all demonstrate the significance of diffusion limitation for removal of soluble substrates in fixed films. Reduction of surface removal rate of the film at low bulk substrate concentration takes place as a half order reaction, as predicted by the model. When the bulk substrate concentration is high enough to ensure full substrate penetration of the biofilm, the removal rate becomes constant, i.e. zero order. Furthermore the distinct change of limiting substrate predicted by the model is experimentally demonstrated (see Fig. 11 and 12). It is noteworthy how well this single concept describes the experimental data, determined under laboratory conditions as well defined as possible. On this basis it can be concluded that there is little ground for introduction of more complicated models for description of the surface removal kinetics - especially when transferred to practical application.

The kinetic parameters calculated according to equation 1 and 2 are given in Table 1.

TABLE 1 Kinetic Parameters for Removal of Soluble Substrates in Fixed Films

| Process | Biofilm Thickness L | Half Order Rate Const. $k_{½a}$ | Intrinsic Zero Order Rate $k_{0f}$ | Diffusion Coefficient D | Temperature T |
|---|---|---|---|---|---|
| | m | $g^{½} m^{-½} s^{-1}$ | $g m^{-3} s^{-1}$ | $m^2 s^{-1}$ | °C |
| | | | NITRATE | | |
| Denitrification | $165 \cdot 10^{-6}$ | $0.73 \cdot 10^{-5}$ | 0.061 | $4.3 \cdot 10^{-10}$ | 22 |
| | 250 - | 0.99 - | 0.21 | 2.3 - | 21 |
| | 560 - | 1.0 - | 0.11 | 5.1 - | 22 |
| | 595 - | 3.0 - | 0.37 | 12.4 - | 22 |
| | | | METHANOL | | |
| | 595 - | 3.4 - | 0.64 | 8.9 - | 22 |
| | | | OXYGEN (Methanol in Excess) | | |
| Oxidation of organics | 100 - | 4.9 - | 1.4 | 8.8 - | 13 |
| | | | OXYGEN (Acetic Acid in Excess) | | |
| | 45 - | 4.8 - | 2.7 | 4.3 - | 10 |
| | | | OXYGEN (Glucose in Excess) | | |
| | 40 - | 4.0 - | 3.0 | 2.6 - | 10 |
| | | | METHANOL | | |
| | 80 - | 2.0 - | 1.3 | 1.5 - | 9 |
| | | | ACETIC ACID | | |
| | 40 - | 4.0 - | 3.5 | 2.3 - | 10 |
| | | | GLUCOSE | | |
| | - | 3.7 - | 6.7 | 1.0 - | 11 |

Note that the rates and coefficients are given for the substrate written with capital letters.

The calculated diffusivities of the soluble substrates are in all cases reduced when compared to values found in pure water given in Table 2. Diffusivities in the range of 15-80% of the values in pure water are found. The difference in reduction of diffusion coefficient for the substrates is assumed to be due to differences in the structure of the biofilm and may in case of charged substrates be caused by electrical interactions between the ions and fixed charges in the biofilm, Riemer and Harremoës (1978).

TABLE 2 Diffusion Coefficients for Some Soluble Substrates in Pure Water

| | Nitrate | Oxygen | Methanol | Acetic Acid | Glucose |
|---|---|---|---|---|---|
| Diffusion Coefficient $D\ 10^{-10}\ m^2\ s^{-1}$ | 15 | 15 | 12 | 12 | 4.7 |
| Temperature T $^oC$ | 25 | 13 | 20 | 25 | 13 |
| Reference | Gray (1972) | Landolt (1969) | Ans (1967) | Perry (1963) | Landolt (1969) |

The intrinsic reaction rates are found to be comparable to findings in the literature. Such data based on direct measurements of the biofilm thicknesses are few and grouped in Table 3. Also literature data enabling calculation of the half order rate constant are few, but available data are collected in Table 4.

TABLE 3 Intrinsic Reaction Rates for Some Soluble Substrates

| Substrate | Intrinsic Reaction $k_{0f}$ $g\ m^{-3}\ s^{-1}$ | Temp. T $^oC$ | Comments | References |
|---|---|---|---|---|
| $C_6H_{12}O_6$ | 2.8 | 21 | Oxidation in a biofilm | Hoehn (1973) |
| | 16.7 | - | Oxidation in a biofilm | Kornegay (1969) |
| | 1.0 | - | Oxidation in a biofilm | Atkinson (1970) |
| | 1.9-13.9 | 22 | Oxidation in a biofilm | LaMotta (1974) (1976A) (1976B) |
| $NO_3$-N | 0.23-0.42 | 21-29 | Denitrifying biofilm | Arvin (1982) |
| $O_2$ | 3.0 | 20 | Oxidation of methanol in a biofilm | Andreasen (1979) |
| | 5.5 | 26 | Oxidation of methanol in a biofilm | Andreasen (1979) |
| | 0.32-0.36 | 25 | Oxidation of nutrient broth in slime from trickling filter | Chen (1981) |

TABLE 4 Half Order Rate Constants for Some Soluble Substrates

| Process | Substrate | Half Order Reaction Rate Constant $k_{½a}$ $g^{½}m^{-½}s^{-1}$ | Reference |
|---|---|---|---|
| Mineralisation | Glucose | $3.8\ 10^{-5}$ | Harremoës(1978A) |
| | Glucose | 4.4 - | Onuma (1982) |
| Denitrification | Nitrate | 3.6 - | Watanabe(1978) Harremöes(1978B) |

The four nitrogen experiments made with the same biofilm at different thicknesses demonstrate in all cases the significance of diffusion limitation. However, the kinetic parameters change as the film grows. The half order rate increased as the film grew older and thicker. This phenomenon is assumed to be attributed mainly to a change of the structure of the film leading to an increase of the diffusion coefficient. Furthermore the intrinsic removal rate of the film changes with the structure. The general trend is increase of the rate with age. Due to varying production of polysaccharides which make up the slimy matrix of the film, the thickness of the film may in periods increase rapidly without a corresponding increase in the number of bacteria. This leads to a decrease of the calculated removal rate based on the total biofilm volume. The present examination of the kinetics of substrate removal in fixed films is not affected by this phenomenon, since the experiments are made within very short periods, where the film properties can be taken as constants. However, the experiments demonstrate the need for combination of kinetics of substrate removal with knowledge of the development and change of the biofilm structure.

Diffusion limitation of the substrates leads to a change of limiting substrate. Fig. 11 and 12 give a direct illustration of the phenomenon for oxidation of acetic acid and for denitrification. For oxidation of acetic acid it is seen that the proportion is about 1.4 g acetic acid/g oxygen. This means that the bulk concentration of oxygen has to be close to the bulk concentration of acetic acid for oxygen not to be limiting. Due to the low solubility of oxygen in water, this requirement will seldom be fulfilled. Furthermore the low solubility means that partial penetration of the biofilm will take place in most cases. The consequence is that often oxygen will be rate limiting to aerobic degradation of organics. Consequently, in practice, the reaction rate will be dominated by half order kinetics governed by the oxygen concentration. This effect of diffusion of the substrate into fixed film is often neglected and may lead to unfavourable design and operation of fixed film reactors.

For denitrification, the change of limiting substrate takes place at a ratio of bulk concentration of methanol to nitrate of about 2 g methanol/g nitrate. This is in close agreement with the value found by Riemer (1977) for denitrification in a submerged down-flow filter. The value is remarkably lower than expected if equal reduction of the diffusivity in the biofilm of methanol and nitrate is assumed. A stronger reduction for the negatively charged nitrate than for the uncharged methanol is proposed to be caused by electrical interaction between the nitrate ions and fixed charges in the biofilm.

## CONCLUSION

A basic concept involving diffusional resistance to the bulk removal rate of soluble substrates in fixed films has been verified experimentally, under well defined laboratory conditions. The results show that a simplified half order - zero order reaction concept adequately describes the phenomena and that more complicated concepts may introduce unnecessary sophistication.

Experiments with denitrification and with oxidation of organics have demonstrated the significance of diffusion limitation for the surface removal of fixed films. The model parameters diffusion coefficients, zero order intrinsic reaction rates and half order rate constants have have been found experimentally.

Diffusion coefficients for nitrate, oxygen, methanol, acetic acid and glucose inside fixed films have been found to be in the range of 15-80% of the values found in pure water.

Zero order intrinsic reaction rates and half order rate constants have been found comparable to the few values cited in the literature.

Oxygen may often be limiting for the surface removal rate of aerobic degradation of organics in fixed films due to the low solubility of oxygen in water and due to diffusional resistance to the oxygen transportation in the film.

Diffusional transportation of nitrate in denitrifying biofilms is found to be reduced as compared to transportation of methanol, supporting a theory of ion-exclusion of the negatively charged nitrate by fixed negative charges in the biofilm.

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