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KINETIC BEHAVIOR OF HETEROTROPHIC AND AUTOTROPHIC BIOFILMS IN WASTEWATER TREATMENT PROCESSES

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ABSTRACT

Conventional laboratory scale annular reactors were employed to investigate the growth dynamics of both heterotrophic and autotrophic biofilms . Based on the experimental observations and physiological aspects, which consist of defining two types of biomass: active biomass (Ma) responsible for substrate removal, and non-active biomass (Md), which plays no role in the biological substrate removal process but is responsible for the observed additional accumulation of biofilms. The experimental results showed that the biological constants were strongly dependent on the influent substrate concentration (So). It was found that the same was true for the volumic substrate removal rate (kov), which shows a surface reaction independent of the film thickness, and that substrate removal in both heterotrophic and autotrophic biofilm reactors remains reactive. The results demonstrated that thinner biofilms ranging from 20 to 30μm have a higher specific removal rate. It is preferable to use thin biofilms for attached culture industrial processes such as three-phase fluidized-bed and turbulent reactors. The results showed that it is possible to eliminate very high carbon loadings up to10 to 15 kgTOD/m^3/d in a three-phase fluidised-bed reactor, and promising results were obtained for simultaneous removal of carbon and nitrogen in the turbulent bed.

KEYWORDS

Heterotrophic biofilm, nitrifying biofilm, growth kinetics, active biomass, non-active biomass, biofilm reactors.

INTRODUCTION

Recently, intensified processes to simultaneously reduce carbon and nitrogen pollution have become more and more important. In such a situation, biological treatment processes using attached cultures are attracting interest as an alternative to conventional treatment processes (suspended activated sludge process for example), considering the advantages of small reactor volumes and high flow rate. Moreover, the immobilization of microorganisms can prevent them from being washed out, and a high sludge age can be more easily maintained.

The engineering of biological treatment processes using attached cultures requires a basic understanding of the kinetic behaviour of the fixed microorganisms responsible for substrate removal from wastewaters. Although much research has been focused on biofilm kinetics, particularly heterotrophic biofilms (La Motta, 1976; Trulear et al, 1982; Belkhadir, 1986; Nguyen, 1989; Harremoës, 1978), little information is available on the dynamics of nitrifying biofilm development.

For many years, the concept of active biofilm thickness has been studied to characterize biofilm behaviours. According to common diffusion-reaction theory, the biofilm accumulation continues until a critical thickness at which the surface removal rate (R) reaches a steady state value, and apparently is not affected by additional biofilm accumulation (Hoehn et al, 1973; La Motta, 1976; Harremoës, 1978; Trulear et al, 1982). However, it must be kept in mind that this concept did not reveal the effect of non-active biomass accumulation. In fact, Liu (1993) found that the variation in the specific substrate removal rate (q) with the film thickness follows an inverse V shape pattern, the maximum q-value being attained at a film thickness of about 25 µm.

Consequently, in order to gain better understanding of the kinetic behaviours of biofilms, we carried out three types of research in parallel, one on anaerobic heterotrophic biofilms (Belkhadir, 1986), the second on aerobic heterotrophic biofilm (Nguyen, 1989), and the last on autotrophic biofilms (Liu, 1993).

Based on the information on either heterotrophic or nitrifying thin biofilms obtained in this study, the practical application of such biofilms to three-phase fluidized-bed (Lertpocasombut, 1991) and turbulent bed reactors (Moreau, 1993) was equally investigated.

FUNDAMENTAL STUDIES ON HETEROTROPHIC AND AUTOTROPHIC BIOFILMS

In order to acquire better knowledge of how heterotrophic and autotrophic biofilms react, several studies were performed by Belkhadir (1986), Nguyen (1989), and Liu (1993) in annular reactors. The results obtained lead to the typical presentation of the different growth phases of a biological film and its substrate removal kinetics, characterized by figure 1.

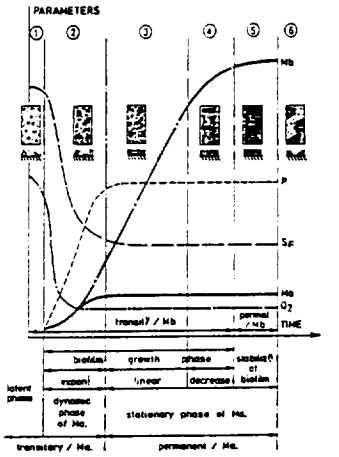


Fig.1. Presentation of different growth phases of biological film (Nguyen, 1989)

Two phases are most important for the comprehension of the biofilm growth and its activity: these are the two stationary regimes with respect to :

- the liquid phase; substrate degradation and product elaboration rates tend towards a maximum value that indicates the achievement of a stationary regime corresponding to a maximum activity of the biofilm,
- the biofilm, which corresponds to the end of the thickening of the biofilm and a balance between active and inactive bacteria making biofilm thicknesses ranging from a few micrometres to several hundred So, there are two types of bacteria that colonize the support at the same time: active bacteria directly concerned with substrate removal and inactive bacteria not concerned with substrate removal because inhibited by fermentative or inhibitory by-products, or by confinement effects connected with production of new cells, which modifies the diffusional transport of the substrate and products around them. These effects can on the whole, be characterized by an apparent affinity constant according to Contois'model (1959).

We must emphasize the fact that colonization dynamics by active and inactive bacteria are closely bound to the available surface of the support. As long as surface is available, concentration in active bacteria increases at the same time as inactive bacteria. As soon as all the surface is colonized, maximal concentration in active

bacteria is reached and substrate removal rate becomes constant. However, biofilm thickness continues to grow because the quantity of active bacteria remains constant but inactive bacteria accumulate, so specific activity decreases very rapidly with biofilm thickness.

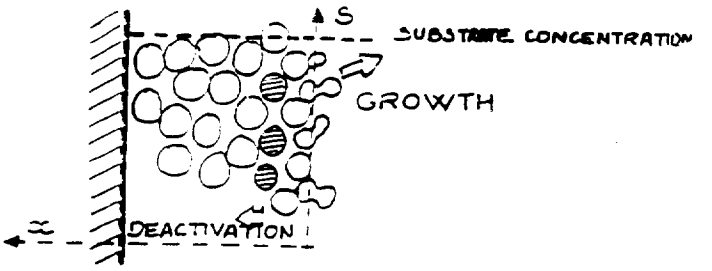


Fig.2. Diagram of bacterial colony development on a support (Belkhadir, 1986)

Concerning the modelling of biofilm growth, the main assumption useful in establishing the equations is directly linked to the structure of the biofilm, which is made of active (Ma) and inactive bacteria (Md). So the total biomass (Mb) is defined as:

$$M_b = M_a + M_d$$

From a kinetics point of view, the growth of an attached culture can be modelled by defining:
- an intrinsic active bacterial growth which is first order with respect to the bacteria concentration:

$$r_{Ma} = \mu_o \cdot M_a \qquad (1)$$

where : M_a : the active mass per unit of surface area (ML^{-2}).

μ_o : the maximum growth rate (T^{-1})

- an intrinsic inhibition rate proportional to the inhibitor concentration and cell density

$$r_{Md} = k_1.I.M_a = k_2 \cdot M_a^2 \qquad (2)$$

where :k_1 : the constant of deactivation ($M^{-1}L^3T^{-1}$)

I : the concentration of inhibitors (ML^{-3})

and we assume that the concentration of inhibitory products is proportional to the concentration of active bacteria.

The rate of accumulation of active bacteria on the support can be described by:

$$(dM_a/dt)_{acc} = r_{Ma} - r_{Md} = \mu_o.M_a - k_2.M_a^2 \qquad (3)$$

Furthermore, the experimental observations of Belkhadir (1986) show that the density of micro-colonies depends on the surface area available on the support. The fraction of the surface covered (a/A_o) is therefore introduced into the kinetic expression, equation (3), bearing in mind that M_a and $(M_a)_{max}$ are proportional, respectively, to the biofilm liquid exchange surface area corresponding to the exchange surface area at the instant t, and to the total exchange surface area at the end of the dynamic phase. We thus arrive at:

$$(dM_a/dt)_{acc} = \mu_o \cdot M_a .B. (A_o-a)/A_o$$

where: a: the surface area covered by micro-colonies at the instant t

Ao : the initial surface area of the support

B : a correction term for the difference between the surface area of the model and that of the physical reality of the phenomenom

By writing substrate balance in transitory regime and as developed in several papers (Bernard, 1990; NATO, 1992) and in numerous articles (see Capdeville and Nguyen, 1990), modelling of aerobic and anaerobic biofilms leads an expression of the volumetric substrate removal rate (k_{ov}) dependent on initial substrate concentration (So) such as :

$$k_{ov} = k_{ov,max} \cdot S_o / (S_o + K)$$

On the contrary, the diffusion-reaction theory developed by several authors (Harremoes, 1978; La Motta, 1976), implies that flow variation is directly linked to the active thickness, because k_{ov} is supposed to be a constant. So, non-active microorganisms are capable of consuming substrate at any time and an increase in initial substrate concentration causes an increase in the active thickness. As demonstrated by Belkhadir (1986), Nguyen (1989) for heterotrophic biofilms and more recently by Liu (1993) for autotrophic biofilms, k_{ov} is not a constant, which means that the inactive bacteria can "see" substrate without being able to consume it. This phenomenon is commonly observed in thin biofilms, which, according to conventional theory, should be totally active.

The theory developed by Capdeville et al (1986-1993) is well confirmed by experiments performed on the three kinds of biofilms mentioned above. Typical curves concerning aerobic heterotrophic and autotrophic biofilms show an agreement with active-inactive bacteria concept, the volumetric substrate removal rate is a function of initial substrate concentration (S_0) (figure 3 (a) and (b)), that growth rate μ_0 is also closely bound to initial substrate concentration (figure 3 (c) and (d)), but that the maximal concentration of active bacteria does not change with S_0 (figure 3 (e) and (f)). In fact, an increase in So has an influence on the biological parameters of the active bacteria but not on the active biofilm thickness as stated by the diffusion-reaction concept.

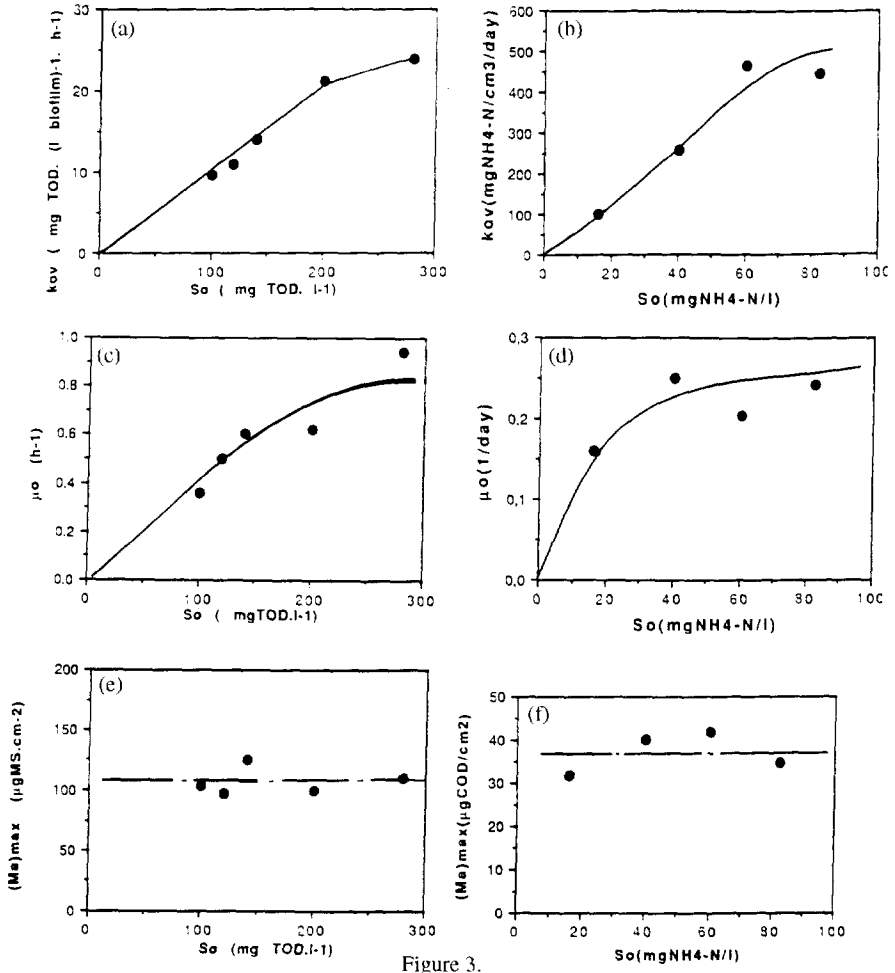


Figure 3.
Influence of So on kov, μo and (Ma)max for heterotrophic (Nguyen, 1989) and autotrophic biofilms (Liu, 1993)

Moreover, regarding biofilm thickness, Liu (1993) has shown that, biofilm thickening is strongly dependent on initial substrate concentration (figure 4). That is to say that, whereas maximal active bacteria concentration remains constant, non-active bacteria accumulate on the support. On the other hand,the specific substrate removal rate (q) reaches a maximum for a biofilm thickness of 25 µm and then decreases because $(M_a)_{max}$ is reached and non-active bacteria accumulate on the support (figure 5).

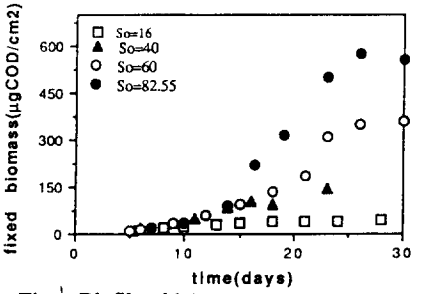


Fig.4. Biofilm thickness evolution versus time.(Liu, 1993)

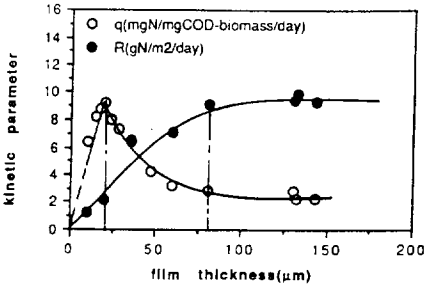


Fig. 5. q variation versus nitrifying biofilm thickness (Liu, 1993)

So, the autotrophic biofilm activity is independent of the thickness but dependent on the surface area to be colonized and only bacteria situated at the liquid/biofilm interface are concerned with substrate removal : the reaction can be considered by a surface reaction.

APPLICATION OF NEW FIXED BIOMASS PROCESSES

Thus, as a result of the fundamental research carried out on heterotrophic biofilms, new granular materials were developed in order to overcome instability problems that occur in a three-phase fluidized -bed reactor. This support known as O.S.B.G. (Optimized Support for Biological Growth, European Patent, 1988) is made up of thermoplastic beads that have undergone surface oxidation and have been positively charged.

These new materials were investigated in a three-phase fluidized-bed reactor for carbon removal. The main results were:

- two stationary regimes : one related to the liquid phase, the other to the biofilm
- very thin biofilms from 15 to 35 µm are capable of removing 9 to 13 $kgCOD.m^{-3}.d^{-1}$
- influence of gas flow on the biofilm retention : the higher the flow rate, the thinner the biofilm without loss of activity (fig. 6). In fact, for an air speed of 5.7 m/h, biofilm accumulates on the support to form a rather thin film, specific activity decreases but TOD removal capacity remains constant. When the air flow is increased, part of the biofilm is detached, thus specific activity measured as INT increases, whereas TOD removal remains constant. So, these results show the influence of shear stresses on biofilm thickness and confirm, as for an autotrophic biofilm, that the specific activity of the biofilm is not linked to its thickness.

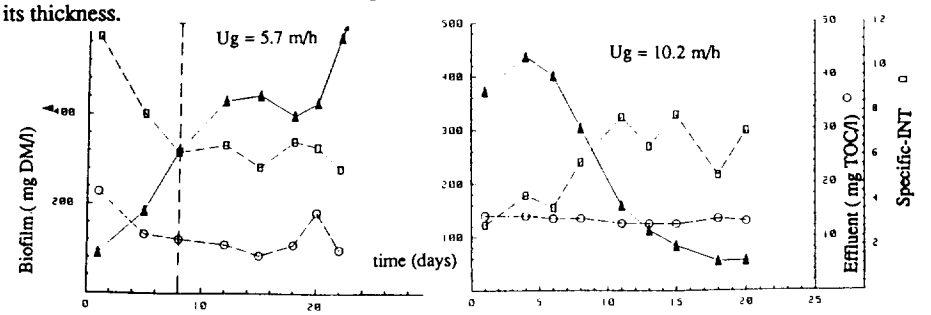
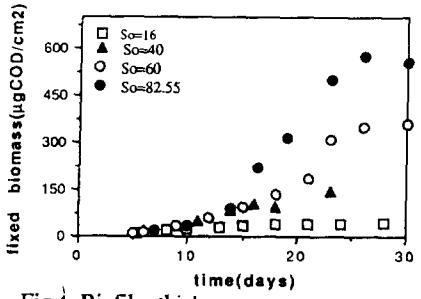


Fig. 6 . Evolution of biofilm activity after an increase of gas flow (5.7 to 10.2 m/h)

We must emphasize the fact that these conditions allow very stable reactor operation and an interestingly low amount of sludge produced (0.11 to 0.23 kg DW/kg soluble COD removed).

To combine the knowledge on heterotrophic and autotrophic biofilms with that on mixed biofilms, O.S.B.G. polystyrene beads (4mm diameter, 1015 kg/m^3) were used in a cubic reactor, similar to an activated sludge basin, fed with a synthetic substrate containing carbon and nitrogen. These beads are moved in the basin thanks only to two linear aeration systems. In such reactors called "turbulent beds",two types of biomass are cultivated: free biomass and biomass fixed on the beads.

Moreover, regarding biofilm thickness, Liu (1993) has shown that, biofilm thickening is strongly dependent on initial substrate concentration (figure 4). That is to say that, whereas maximal active bacteria concentration remains constant, non-active bacteria accumulate on the support. On the other hand,the specific substrate removal rate (q) reaches a maximum for a biofilm thickness of 25 µm and then decreases because $(M_a)_{max}$ is reached and non-active bacteria accumulate on the support (figure 5).



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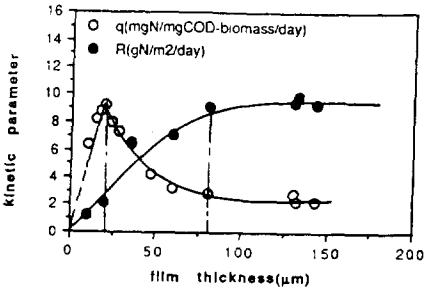

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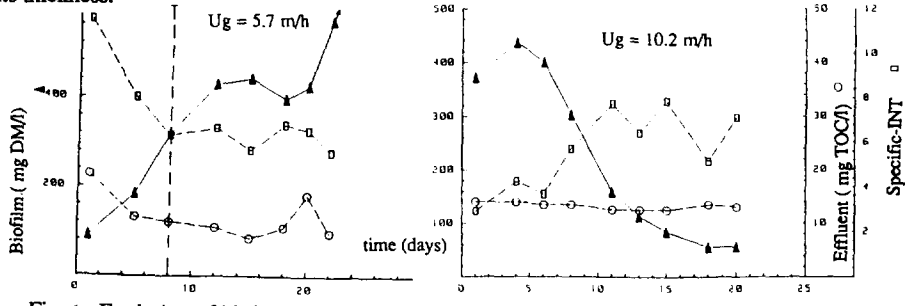

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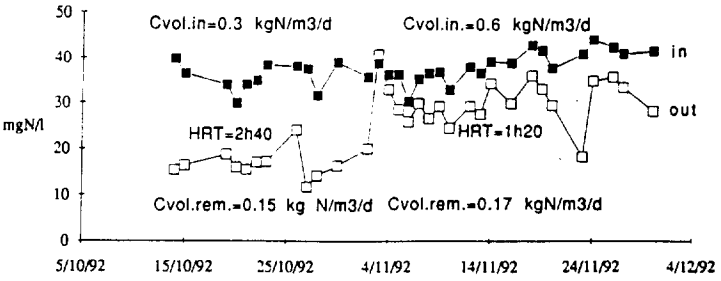

Fig. 9. Nitrogen concentration at the inlet and outlet of the turbulent bed for two different hydraulic retention times (Moreau, 1993).

So, we are faced with a totally active biofilm, on which the selection pressure due to shear stresses always erodes new cells that develop on the support and so control of the biofilm thickness and the increase of nutrient removal capacity, are very difficult. Thus, new granular materials are at present being made in our laboratory. They are mechanically treated to have a rougher surface and they will be tested in various new wastewater fixed biomass treatment processes at laboratory scale.

CONCLUSION

The fundamental studies performed on the growth kinetics of heterotrophic and autotrophic biofilms have shown two permanent regimes : one with respect to the liquid, the other with respect to the biofilm. The new concept developed distinguishes two types of bacteria, active and inactive, that colonize the support at the same time. The active bacteria (Ma) are situated at the biofilm / liquid interface and are responsible for metabolizing substrate, whereas the de-activated bacteria (Md), located inside the biofilm, are responsible for its observed accumulation. Moreover, it should be stressed that the behaviour of autotrophic biofilm is similar to that of the heterotrophic one and that development of a thin and active nitrifying biofilm is possible under constant and well known shear forces. Moreover, such attached biomass systems show a very strong dependence between the biological parameters, characteristic of the bacterial growth (μ_o), and initial substrate concentration. This is notably seen from the increase in the volumetric substrate removal rate (k_{ov}) with S_o. Then, whatever the biofilm thickness, the reaction tends towards a surface reaction. Thus, it is necessary to first control the attachment capacity of microorganisms as regards the support and secondly, to control the active thickness to obtain the best performance of a biological system without accumulating too many bacteria. Successful applications, notably in the three-phase fluidized-bed reactor, have been achieved , and our research team is now trying to understand the dynamic growth of mixed biofilms better.

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