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Membrane attached biofilms for waste treatment—fundamentals and applications

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Abstract: Membrane Attached Biofilms (MABs) are being used in an increasing variety of bioreactors. Extractive Membrane Bioreactors (EMB) have been developed at Imperial College (1,2) for the aerobic biotreatment of toxic organics which employ MABs for treating Volatile Organic Compounds (VOCs)-containing wastewaters without incurring air-stripping problems. Investigations of the key factors controlling the optimal operating conditions for the EMB system have shown that process efficiency is highly dependent on the development of these MABs. Therefore MAB development and its influence on the flux across the membrane over time has been studied and is presented here. Two MAB model systems have been studied; *Xanthobacter autotrophicus* GJ10 growing on 1,2-Dichloroethane (DCE) and *Pseudomonas* JS150 growing on Monochlorobenzene (MCB). The results show that there is a problem in this system with excess biofilm growth on the membrane surface, resulting in reduced flux of organic substrate across the membrane. At the same time, a diffusion-reaction model has been developed to explain the experimental results, and to describe the behaviour of the EMB. It was theoretically concluded that an optimal biofilm thickness could be found from a compromise between the level of air-stripping and flux of pollutant across the membrane, and that cell endogenous decay could be used to manipulate the biofilm thickness. Methods of controlling excessive growth of biomass have been investigated, and the addition of sodium chloride to the biomedium to control excessive biofilm development has been shown to be effective.

INTRODUCTION

Work carried out over the last 3 years in the Department of Chemical Engineering at Imperial College has utilised MABs for the aerobic biotreatment of toxic Volatile Organic Compound (VOC) containing wastewaters (3). By allowing biofilms to spontaneously attach to the surface of a silicone rubber membrane, it is possible to supply oxygen and VOC from opposite sides of the biofilm, thus eliminating the air stripping common in conventional bioreactors (4). The wastewater stream and the biomedium are separated by a silicone rubber membrane as shown in Figure 1. Poorly water-soluble organic compounds pass rapidly through the non-porous membrane and are biodegraded in the biological side, whilst inorganic species in the wastewater stream cannot. The ionic components of the wastewater thus have no effect on the degradative microorganisms.

Important parameters in predicting the behaviour of biofilm/immobilised cell reactors are the thickness of the biofilm, the rate of growth of the biofilm, the diffusion coefficients of substrates and products in the biofilm, and the density of the biofilm. In common with other workers investigating biofilm/immobilised cell systems is the difficulty of how to determine fundamental biofilm properties.

Most biofilm thickness measurement techniques described in the literature require removal of the biofilm from the system, are destructive, and in addition often necessitate some form of sample preparation with consequent changes in properties (5,6,7). A novel technique for measuring the thickness of growing MABs *in situ* has been developed at Imperial College (8), opening up possibilities for making new measurements of fundamental biofilm properties. This simple technique, based around simultaneous projection and magnification of the image of a MAB grown in a Single Tube Extractive Membrane Bioreactor (STEMB), offers advantages over the considerably more complex alternatives described in the literature. It allows biofilm thickness to be measured over time, without disrupting bioreactor operation.

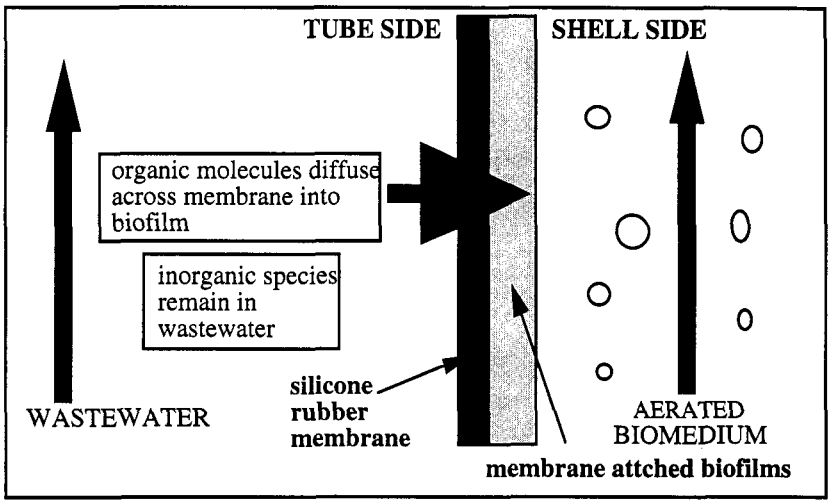

Fig. 1 Principle of operation of the EMB.

Although the presence of biofilms is essential to prevent air-stripping, if these biofilms grow excessively this causes problems, both hydraulically, since the biomedium can no longer flow freely around the shell side of the module, and through its effect on the mass transfer of the organic compound from the wastewater to the biomedium. Therefore thick biofilms have high mass transfer resistances which reduce the flux of pollutant across the membrane, whilst thin biofilms lead to high levels of air stripping.

At larger scales, the production of superfluous biomass is undesirable since the resulting sludge must be treated and disposed of. Previous work in this department, using chemostat cultures, has shown that the addition of high concentrations (2-5%) of sodium chloride to the biomedium reduces the cellular yield. This is because sodium ions can freely enter the bacterial cell and must be actively pumped out across the cell membrane to negate their effect on osmotic pressure and reduce their inherent toxicity. This process expends energy, which is obtained from the carbon source, with the subsequent production of carbon dioxide. This carbon is therefore unavailable as a growth substrate and hence the growth yield of microorganisms is reduced. Two different microorganisms were used in these experiments; a pseudomonad and a *Xanthobacter* species. They have been selected as model systems to study the influence of biofilm accumulation on process performance over time, and also to study the influence of addition of sodium chloride on the control of excessive biofilm growth.

Few studies have focused on developing mathematical models to describe these MABs. Wanner and Gujer (1986) (9) proposed a multispecies biofilm model which has successfully described the behaviour of biofilms used for oxygenation of biofilm reactors such as the ones proposed by Wanner et al. (1994) (10) and Wilderer (1995) (11). Pavasant et al. (12) developed a dynamic model that describes the dynamic biofilm accumulation and overall system operating performance. The biofilm model is derived in cylindrical co-ordinates and is able to predict several variables simultaneously, i.e. biofilm thickness, pollutant flux across the membrane, organic and suspended biomass concentrations in the biomedium, the rate of carbon dioxide production, and organic concentration in the gas outlet.

In the present paper the model simulations are compared with experimental results for a *X. autotrophicus* MAB growing on DCE. The simulations describe the changes in biofilm thickness, DCE flux across the membrane and the effect of sodium chloride addition on microbial growth in the STEMB.

MATERIALS AND METHODS

Pseudomonas sp. strain JS150 is capable of utilising monochlorobenzene (MCB) as a sole carbon and energy source and was obtained as freeze-dried culture from J.C. Spain of the Air Force Civil Engineering Support Agency, Florida, USA (13). *Xanthobacter autotrophicus* GJ10 (14) is capable of using 1,2-dichloroethane (DCE) as a sole carbon and energy source and was obtained from D. Janssen of the University of Groningen. The nutrient salts medium composition is given by Janssen et al. (1984) (15).

MCB and DCE concentrations in the wastewater, exit gas and biomedium were analysed using a Perkin Elmer Gas Chromatograph with a flame ionisation detector and a megabore column 25 m long and 0.23 mm i.d. with BP1 (SGE, Australia) as the stationary phase. 1 µl samples were injected directly onto the column. The temperature program ran from 40 _C to 120 _C. Peak areas were compared with those of solutions of known MCB or DCE concentration. The uncertainty in this assay (quoted as the standard deviation of three separate determinations at the 100 mg L^{-1} level) was 4.2 %. The detection limits were 0.2 mg L^{-1} MCB and 0.1 mg L^{-1} DCE. The exit gas sample was also analysed in this way with a sample volume of 1 ml.

STEMB Layout

The layout of the STEMB is shown in Figure 2. An airlift bioreactor was coupled to a membrane module via a recirculating biomedium flow. Dissolved oxygen concentration was kept above 20 % by varying the air flow rate. pH was maintained at 7.0 ± 0.05 and temperature at 30 _ C ± 0.1. The square glass module used in this work had external dimensions of 200 mm height x 60 mm x 60 mm, with a glass thickness of 5 mm. A single silicone rubber tube (2.0 mm i.d. x 0.5 mm wall thickness), supplied by Esco Rubber (UK) Ltd, was fixed vertically along the centre of the module. Wastewater was pumped through the insides of the tube and biomedium was recirculated around the shell side of the module. Tubing throughout the system was constructed from teflon in order to minimise the losses of MCB and DCE through the tubing walls. Connections between sections of teflon were made using flexible viton tubing. Nutrients were provided continuously to the biomedium at a rate of 0.069 L h^{-1} and the excess biomedium left the reactor at the same rate. NaCl was added to the biomedium at different stages to make 2% NaCl.

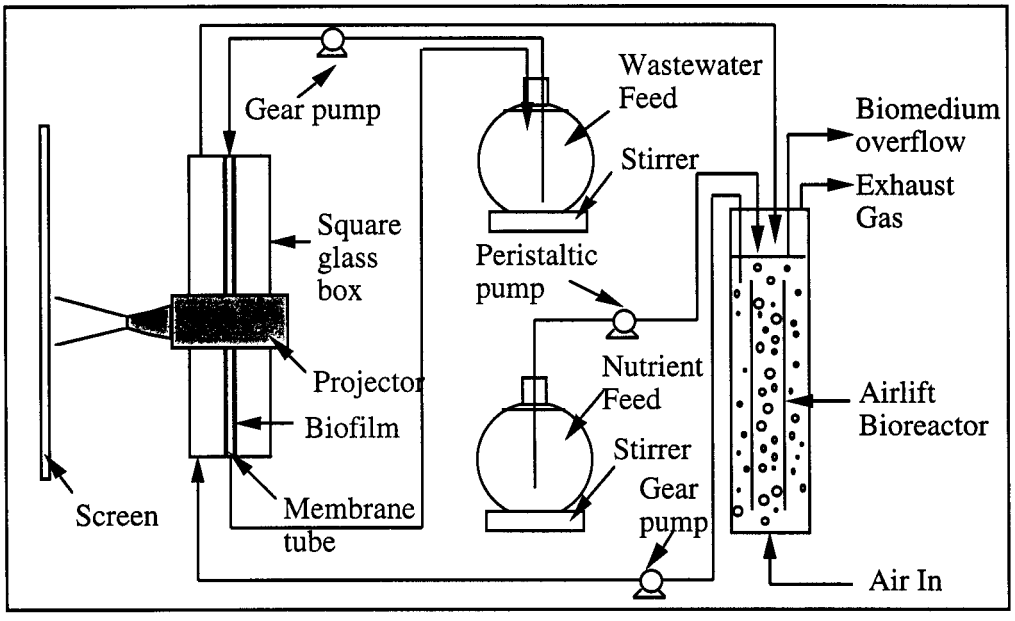

Fig. 2 Schematic layout of the Single Tube Extractive Membrane Bioreactor (STEMB).

Projection technique (PT) for biofilm thickness measurement

The image of the silicone rubber membrane tube with attached biofilm is projected onto a screen with an attached scale, as shown schematically in Figure 2. The projector (an Aldis Tutor 1000 (Aldis Bros. Ltd., UK) fitted with a 240V/1000 Watt projector lamp) was placed at a distance of 4.0 m from the screen. The diameter of tube plus attached biofilm was measured directly on the screen from this projected image using the attached scale - the maximum resolution obtainable with the 1 mm divisions led to an estimated error in biofilm thickness of ± 10 µm. After each experimental run, the biomass attached to the membrane tube was removed, dried in an oven at 110 °C, and weighed. The average density of the membrane-attached

biofilm can then be determined from the average value of biofilm thickness and the quantity of dry biomass.

BIOFILM MODEL EQUATIONS

The mathematical model for the STEMB was developed to describe the model system *X. autotrophicus* GJ10-DCE and it is based on the following assumptions:

1. It assumes that the biomass grows following dual Monod kinetics (μ_X) in oxygen and DCE concentrations (3). The kinetic parameters of the immobilised cells in the biofilm are assumed to be identical to those of freely suspended cells. Cell maintenance and death is described using the concept of cell decay, and an endogenous decay coefficient, μ_e. Mathematically, cell maintenance and endogenous decay are related and they can be inter-converted as follows: in the presence of NaCl, microorganisms may consume more substrates to produce more maintenance energy to pump sodium ions out of the cells; on the other hand, microorganisms may consume more substrates and produce some cellular components which later break down to give more energy for pumping the excess sodium ions out of cells; a process of cell endogenous decay.

The specific reaction rates of DCE, oxygen and carbon dioxide can be written as functions of μ_X and μ_e with their corresponding yield coefficients.

$$\mu_s = -\left(\frac{\mu_x}{Y_{x/s}}\right) \tag{1}$$

$$\mu_o = -\left(\frac{\mu_x}{Y_{x/o}} + \mu_e Y^e_{o/x}\right) \tag{2}$$

$$\mu_c = \left(\frac{\mu_x}{Y_{x/c}} + \mu_e Y^e_{c/x}\right) \tag{3}$$

2. The detachment rate is assumed to be dependent on biofilm density (r_{bf}), biofilm thickness (d) and shear force (g) following the model proposed by Rittmann (1982) (16).

$$r_d = k_d \rho_{bf} (R_{in}\delta)\gamma^{0.58} \tag{4}$$

The attachment rate of suspended biomass to the biofilm is assumed to be a first order function of suspended biomass concentration.

$$r_a = k_a P_{x,sh} \tag{5}$$

Hence the net removal rate of biofilm is:

$$\Gamma_{net} = r_d - r_a \tag{6}$$

3. The STEMB has been operated at a high wastewater flow rate in the tube side so it is reasonable to assume a constant DCE concentration on the inside of the membrane tube throughout its length.

4. The mass transport of DCE, oxygen and carbon dioxide in the biofilm can be described by Fick's law.

5. The biomedium is well mixed throughout the membrane module and bioreactor.

6. Biofilm density, and the diffusivities and mass transfer coefficients of DCE, oxygen and carbon-dioxide are constant over the range of operating conditions.

7. The biofilm is homogeneous and has a smooth surface.

Hence, the model equations for DCE, oxygen and carbon dioxide in the biofilm can be formulated as:

$$\frac{\partial P_{s,bf}}{\partial t} = \frac{\vartheta_x}{r}\frac{\partial}{\partial r} r \frac{\partial P_{s,bf}}{\partial r} + \mu_{s,bf}\rho_{bf} \tag{7}$$

$$\frac{\partial P_{o,bf}}{\partial t} = \frac{\vartheta_o}{r}\frac{\partial}{\partial r} r \frac{\partial P_{o,bf}}{\partial r} + \mu_{o,bf}\rho_{bf} \tag{8}$$

$$\frac{\partial P_{c,bf}}{\partial t} = \frac{\vartheta_c}{r}\frac{\partial}{\partial r} r \frac{\partial P_{c,bf}}{\partial r} + \mu_{c,bf}\rho_{bf} \tag{9}$$

subject to the following boundary conditions:

$$\vartheta_s \left.\frac{\partial P_{s,bf}}{\partial r}\right|_{R_{in}} = -k_{R_{in},s}\left(P_{s,t} - P_{s,bf}\big|_{R_{in}}\right) \tag{10}$$

$$\vartheta_o \left.\frac{\partial P_{o,bf}}{\partial r}\right|_{R_{in}} = 0 \tag{11}$$

$$\vartheta_c \left.\frac{\partial P_{c,bf}}{\partial r}\right|_{R_{in}} = 0 \tag{12}$$

$$\vartheta_s \left.\frac{\partial P_{s,bf}}{\partial r}\right|_{R_{out}} = k_{R_{out},s}\left(P_{s,sh} - P_{s,bf}\big|_{R_{out}}\right) \tag{13}$$

$$\vartheta_o \left.\frac{\partial P_{o,bf}}{\partial r}\right|_{R_{out}} = k_{R_{out},o}\left(P_{o,sh} - P_{o,bf}\big|_{R_{out}}\right) \tag{14}$$

$$\vartheta_c \left.\frac{\partial P_{c,bf}}{\partial r}\right|_{R_{out}} = k_{R_{out},c}\left(P_{c,sh} - P_{c,bf}\big|_{R_{out}}\right) \tag{15}$$

The rate of change of biofilm thickness is a function of the total growth rate and the total rate of biofilm removal.

$$\frac{dV_{bf}\rho_{bf}}{dt} = \int_{R_{in}}^{R_{out}} \overline{\mu}_{x,bf}\rho_{bf}(2\pi r L)dr - \Gamma_{net} 2\pi R_{out} L \tag{16}$$

The dynamic model involves the growth of biofilm, leading to a non-stationary biomedium-biofilm interface at which the oxygen and DCE mass transfer take place. This forms a moving boundary problem and the Front-Fixing method described by Crank (1984) (17) is employed to transform the equations to a stationary boundary system. The final set of equations are discretised using the finite difference method, and solved via general PROcess Modelling System (gPROMS) version 1.4c, developed in the Centre for Process Systems Engineering at Imperial College (Barton and Pantelides, 1994) (18).

RESULTS AND DISCUSSION

Projection Technique (PT) for Biofilm Thickness Measurement

Figure 3 shows the evolution of biofilm thickness over time for the two model biofilm/immobilised systems studied. This two model systems show completely different development modes. *X. autotrophicus* GJ10 biofilm thickness increased faster and even after 22 days did not seem to reach a steady state while *Pseudomonas* sp. JS150 biofilm growth seems to taper off after 10-12 days. Another difference in the systems was the evolution of suspended biomass. While in the first model system the initial high turbidity of the biomedium disappeared after 5-6 days and biomass seemed to settle attached to the membrane tube, in the second system the turbidity did not decrease significantly over the entire period of operation. The biofilm density was calculated for both systems and gave an average of 60 kg m^{-3} and 95 kg m^{-3} for the first and second systems respectively. These results are within the range of biofilm densities reported in the literature, which normally vary between 10 and 130 kg m^{-3} (19).

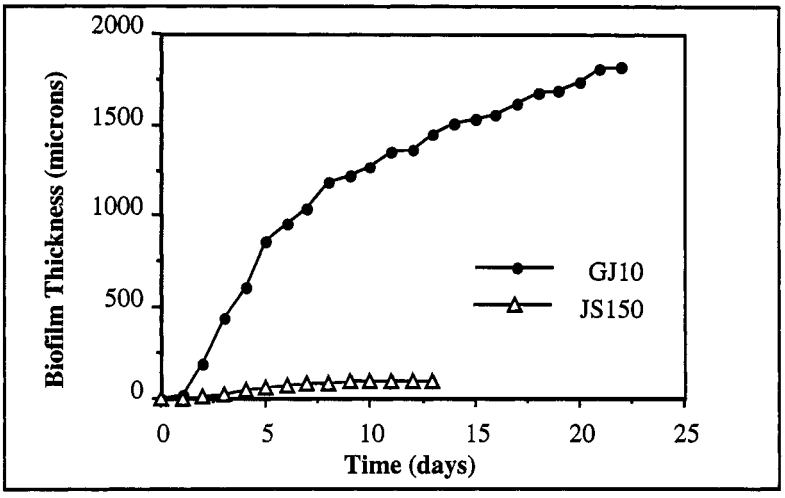

Fig. 3 Evolution of biofilm thickness over time for the two model systems studied.

STEMB operation

Figure 4 shows MCB flux and biofilm thickness over time for *Pseudomonas* sp. strain JS150. Over the time span of this experiment, no reduction in MCB flux can be discerned. This seems to be because of the slow growth rate of the *Pseudomonas* biofilm, which never reached sufficient thickness to cause flux reduction. The addition of sodium chloride to the system would therefore show no improvement and thus the experiment was repeated using *X. autotrophicus* GJ10. Figure 5 shows the DCE flux and biofilm thickness evolutions. There is a marked decrease in the DCE flux with biofilm thicknesses higher than 150 microns.

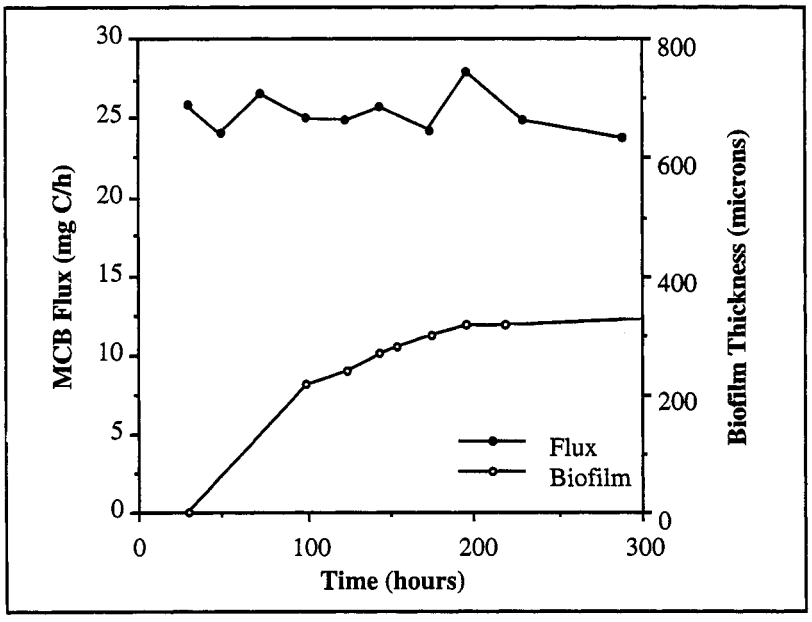

Fig. 4 MCB flux and biofilm thickness over time for *Pseudomonas* JS150: [NaCl] = 0 mg / L

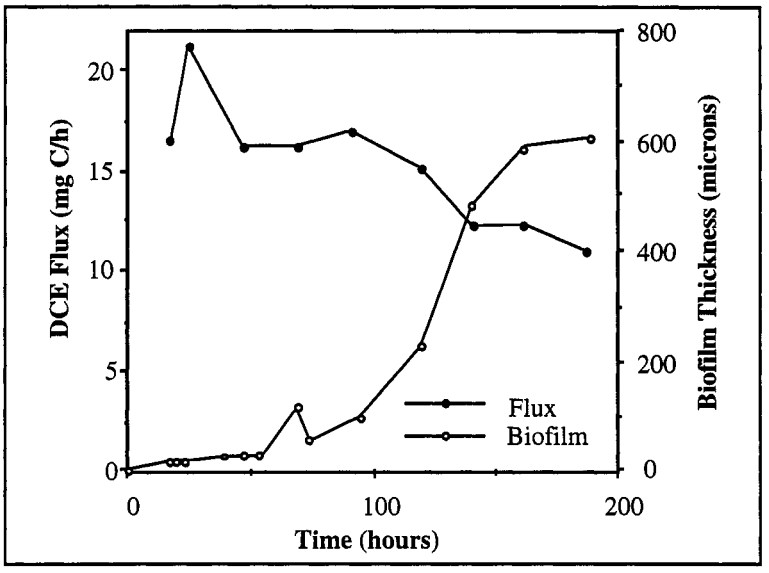

Fig. 5 DCE flux and biofilm thickness over time for *Xanthobacter* GJ10: [NaCl] = 0 g / L

Figure 6 shows the DCE flux and the biofilm thickness over time in the STEMB for *X. autotrophicus* GJ10 and 30 g L^{-1} sodium chloride. After approximately 200 hours with no sodium chloride present in the biomedium the flux of DCE across the membrane had dropped to around 12 mg C h^{-1} (Figure 5). When sodium chloride was present in the biomedium at 30 g L^{-1}, the DCE flux across the membrane after 200 hours remained close to 20 mg C h^{-1} (Figure 6). These results suggest that the addition of sodium chloride to the biomedium can indeed be useful for controlling the rate of biofilm growth.

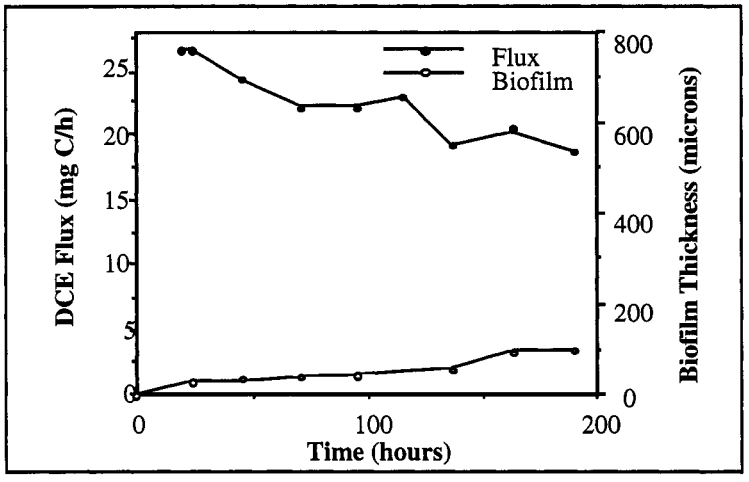

Fig. 6 DCE flux and biofilm thickness over time for *Xanthobacter* GJ10: [NaCl] = 30 g / L

Figure 7 shows the evolution of the DCE air-stripped from the biomedium into the exhaust gas of the bioreactor. Considering these results from the perspective of maximising DCE flux, it is obvious that biofilms above 200 microns are undesirable. It seems from Figure 5 that the optimal lies in the region of 100-150 microns. However looking at the results in Figure 7 it seems that a biofilm of approximately 250-300 microns is necessary to avoid air-stripping from the bioreactor. Therefore there is a "no air stripping constraint" on biofilm thickness which reflects the need for biofilm growth to minimise air stripping in the EMB system. In order to improve the performance of the EMB it was necessary to find a way of limiting excessive growth.

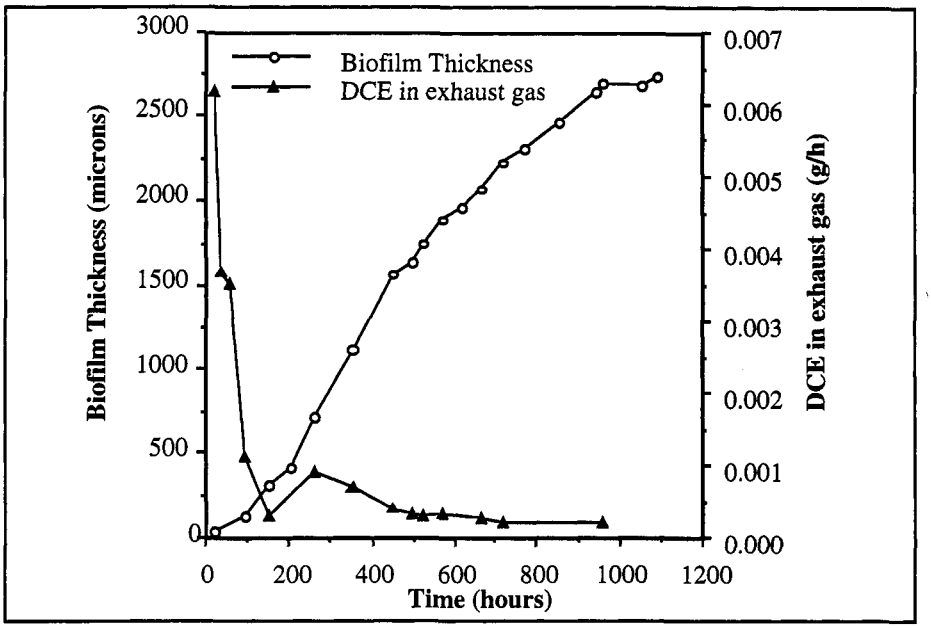

Fig. 7 Evolution of biofilm thickness and DCE air stripping losses over time.

Model verification

A comparison of the experimental biofilm thickness profile for the *X. autotrophicus* GJ10 system with the simulation result is shown in Figure 8. Two sets of experimental data for this model system are plotted and are referred to as Run1 and Run2. The mathematical model is able to predict the increasing trend in biofilm thickness and agrees well with the data from experimental Run 2, in which a biofilm thickness of around 0.7 mm was recorded after 10 days of operation. All of the curves (experimental and simulated) shown in Figure 8 indicate that the biofilm thickness increases continuously over the whole period, and shows no sign of reaching steady-state over 20 days of operation. It would be interesting to solve the same mathematical model for the *Pseudomonas* JS150 system and verify if a steady-sate is predicted. However further experiments are required in order to calculate the kinetic parameters for this system.

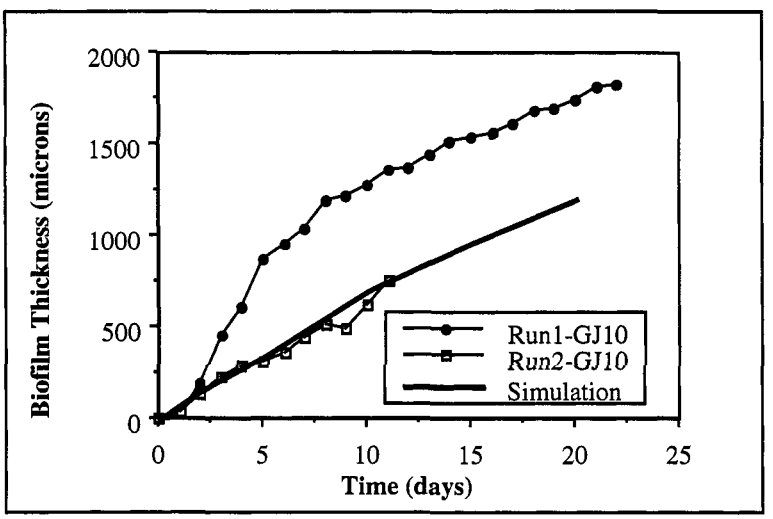

Fig. 8 Comparison between the experimental data with the simulation results for the *X. autotrophicus* GJ10 model system.

Experiments performed in the STEMB with *Xanthobacter autotrophicus* GJ10 grown on DCE in an environment containing 2% w/v NaCl have shown that it is possible to significantly reduce the biofilm growth rate, preventing the previous drop in the flux of DCE. Figure 9 shows the results of growing a biofilm with 0% NaCl, then adding 2% NaCl, and then removing the NaCl from the biomedium again. During the period of addition of NaCl there is stabilisation of biofilm growth and also of the decrease in DCE flux. As soon as the NaCl is removed from the biomedium the flux drops and the growth of biofilm becomes exponential. These results suggest that the addition of NaCl to the biomedium can indeed control the rate of growth of a biofilm. The biofilm can then be controlled in order to achieve a thickness sufficient to avoid air-stripping but at the same time hold flux relatively high. The model slightly over estimates the biofilm thickness, but the overall trend is correctly predicted. This agrees with chemostat experiments which showed that for several different microorganisms (including *X. autotrophicus*), in a suspended growth system, increased sodium chloride concentration resulted in increased maintenance requirement. The gradual decline in DCE flux across the membrane tube with an increase in biofilm thickness is correctly predicted as is the levelling off of biofilm growth in the presence of NaCl.

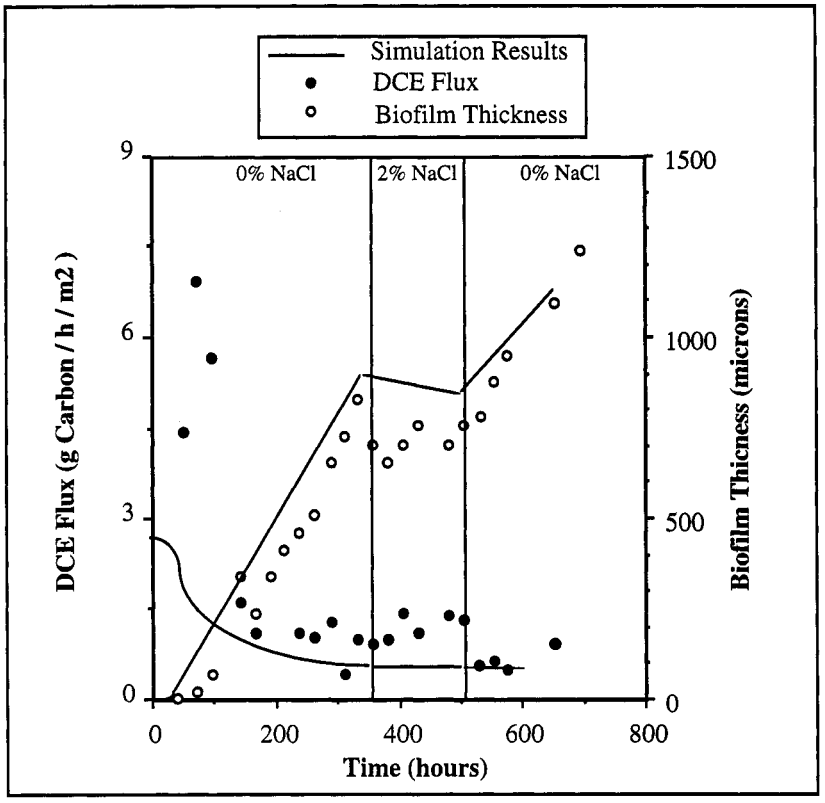

Fig. 9 Comparison of experimental data on DCE flux and biofilm thickness with simulation results.

CONCLUSIONS

The projection technique allows rapid, non disruptive measurements of biofilm thickness to be made over time and it can be employed to study a wide range of membrane materials and microbial cultures, and the development of biofilms under different environmental conditions to be studied.

In the presence of a thin biofilm on the surface of the membrane tubes, air-stripping of the organic compound occurs, since the compound is degraded for the most part in the aerated region of the bioreactor. However if the biofilm is thick it causes a marked decrease in the flux, upsetting performance of the EMB system. A trade-off is therefore necessary between air-stripping and flux reduction.

Current research in this group is investigating the use of sodium chloride to prevent further growth in a biofilm already present on the surface of the membrane - that is, to hold the biofilm thickness at a level

where air stripping and flux reduction are both low. The results presented here suggest that sodium chloride addition may indeed be a cheap, non-toxic, effective method for controlling biofilm growth in a full scale EMB. This could greatly enhance the performance of such a reactor, due to the maintenance of a high transmembrane flux of organic pollutant. It would also increase the time for which the membrane module could be in use before cleaning of its surface became necessary. The slow growth rate of *Pseudomonas* JS150 biofilm suggests that the addition of sodium chloride may not always be necessary in order to maintain a high flux in an EMB system. However at larger scales, running for significantly longer periods, it is likely that excessive growth of this organism will also eventually cause problems in the EMB.

Application of this technique to other wastewater treatment systems, both biofilm and suspended growth, could also lead to important enhancements of treatment processes through the reduction of sludge production. Further work is necessary to determine whether this biomass reduction technique is applicable to mixed populations of bacteria. Different bacteria will tolerate different maximum concentrations of sodium chloride and therefore a salt concentration which reduces the growth rate of one strain may be sufficient to result in the death of another strain, so altering the balance of populations in the mixed culture.

The mathematical model presented gives an overall description of the phenomena occurring in the STEMB and in particular gives good predictions for trends in biofilm thickness profiles. The behaviour of the STEMB under the presence of NaCl was simulated and tested experimentally and it was found that model predictions compare favourably with experimental data. Therefore, with a future availability of specific rates of endogenous decay at different levels of NaCl, the model can be used to predict the NaCl strength in the biomedium that gives an optimal biofilm thickness in the EMB . This mathematical model is not only used to compare with experimental results but can be used as a predictive tool for future design and operation of larger scale EMBs.

ACKNOWLEDGMENTS

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NOMENCLATURE

k Mass transfer coefficient (m s^{-1})
k_a Attachment rate coefficient (m s^{-1})
k_d Detachment rate coefficient (-)
L Length of the membrane tube (m)
P Concentration (kg m^{-3})
r Radius (m)
R Radius on the membrane module (m)
V Volume (m^3)
Y Yield coefficient (-)

Symbols

d Dimensionless thickness (-)
G Overall rate of removal (kg m^{-2} s^{-1})
g Shear stress (kg m^{-1} s^{-2})
J Diffusivity (m^2 s^{-1})
m Specific reaction rate (s^{-1})
r Biofilm density (kg m^{-3})
t Dimensionless time (-)

Subscripts

bf	Biofilm
biom	Biomedium
c	Carbon dioxide
g	Gas
i	Initial
o	Oxygen
R_m	Inner diameter of membrane tube
R_{in}	Outer diameter of clean membrane tube
R_{out}	Outer diameter of membrane tube including biofilm thickness
s	DCE (carbon source)
sh	Shell-Side of membrane module
t	Tube-Side of membrane module

Superscripts

e	Endogeneous decay

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