Use of a membrane flow cell to measure average mass transfer coefficients in denitrifying biofilms

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Abstract
Average mass transfer coefficients within denitrifying biofilms were determined during biofilm growth with an inert compound (LiCl) in a membrane flow cell at different flow conditions, until the biofilm reached (pseudo-) steady state. Similar values were obtained for the steady-state mass transfer coefficients of LiCl within the different biofilms. However, those coefficients were higher during the transient period of biofilm growth for the biofilm developed under the highest upflow velocity.
Key words Denitrifying biofilm; flow cell; mass transfer coefficients

Introduction

Mass transfer inside biofilms is often the process limiting substrate consumption rates in biofilm reactors. Local diffusivities within biofilms have been measured by sophisticated direct methods such as microelectrodes and FRAP-Fluorescence Recovery After Photobleaching (Beyenal and Lewandowski, 2000; Bryers and Drummond, 1998). More often, average diffusivities were determined by less expensive indirect methods based on the measurement of substrate consumption rates together with the use of diffusion-reaction biofilm models (Harremoës, 1978), but the results are dependent on the appropriateness of the mathematical model itself. Usually, these models consider molecular diffusion as the only solute transport mechanism, although in natural and industrial biofilms convective and molecular diffusion were found to co-exist in many cases (De Beer *et al*., 1996). By measuring mass transfer coefficients within biofilms, values of a transport parameter are obtained that include all such mechanisms, thereby yielding an overall measure of the actual mass transfer rates. To evaluate the average mass transfer coefficients within biofilms, a simplified method can be used, based on a mass balance applied to the transport of a non reactive tracer through the biofilm formed on a porous membrane (Vieira *et al*., 1993; Brito and Melo, 1999).

The present work was carried out to monitor the average mass transfer coefficients in a denitrifying biofilm grown in a membrane flow cell, using different upflow velocities, in the laminar flow range, similar to the ones used in denitrifying biofilm reactors.

Materials and Methods

Experimental system

A denitrifying biofilm was grown in a vertical flow cell (Figure 1) consisting of two chambers or compartments (I and II), separated by a hydrophilic membrane of cellulose esters, with a mass transfer area and pore diameter of $1.6 \cdot 10^{-3}$ m^2 and 0.22 μm, respectively. The flow cell was made of plexiglass with a semi-circular cross-section geometry. A centrifugal pump was connected to each chamber (circuit I and circuit II) in order to recycle the liquid continuously. Sample ports were placed in chambers I and II.

Three experiments were carried out by applying three different upflow velocities to the system. Upflow rates of 0.004 $m \cdot s^{-1}$ (Biofilm 1), 0.01 $m \cdot s^{-1}$ (Biofilm 2) and 0.04 $m \cdot s^{-1}$ (Biofilm 3) were adjusted with a flow meter on both sides of the membrane. A differential manometer was connected to both sides of the membrane in order to ensure the same pressure and avoid the transport due to a pressure gradient across the membrane. The total volume of each circuit was about 800 mL for the two first cases and 500 mL for the last case.

Initially, circuit I was inoculated with a denitrifying bacterial suspension and water was pumped into circuit II. After 24 h the inoculum was replaced by medium solution. Biofilm1 and 2 worked in fed-batch mode to promote biofilm growth during the experiments. Biofilm 3 was fed in a continuous mode, working with 4 hours of hydraulic retention time.

The nitrate concentration in the feed of the flow cell was always 50 mg N/L. A C/N ratio of 4, using acetate as carbon source, was used for Biofilms 1 and 2, while a C/N of 2.25, with methanol as carbon source, was used for Biofilm 3. Inorganic elements were used as nutrients. The pH was adjusted between 7.3 and 7.5. The operating temperature was 20 °C.

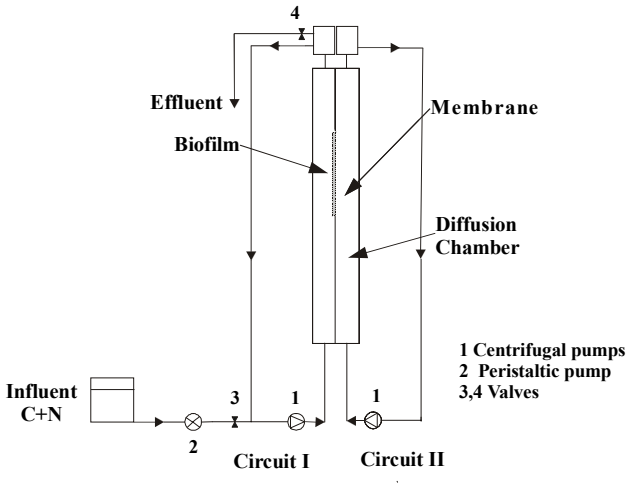

Figure 1 Mass transfer flow cell.

Mass transfer measurements within the biofilm were performed by introducing an inert compound (lithium chloride) in the membrane flow cell both without biofilm (clean membrane) and during biofilm growth (Vieira *et al*., 1993; Brito and Melo, 1999). A fixed amount of LiCl (200 mg $Li^{+}\cdot L^{-1}$) was added to vessel I together with the medium. The mass transfer experiment started after equilibrium conditions were reached. Samples were collected at intervals of 30 min, during 8 h, in both circuits. After that, LiCl was removed from the system. Lithium concentration was measured by atomic absorption spectroscopy (Varian SpectrAA.250 plus). Biofilm adsorption studies were also carried out to verify whether there was significant lithium adsorption on the biofilm during the mass transfer measurements.

Biofilm characterization

At the end of the experiments, the average biofilm thickness was determined in Biofilms 1 and 2 with a digital micrometer and a video camera, according to Brito and Melo (1999). In the case of Biofilm 3, the average biofilm thickness was determined with a microscope Leica Leitz DMRD at magnification of 5x0.12p and with a calibrated ocular micrometer.

The biofilm was detached from the support by ultrasound treatment. The following parameters of the biofilm were characterized: Total Proteins and Total Polymers according to the methods of Lowry (Sigma kit 5656) and Dubois (1956), respectively; dry weight (TS) by Standard Methods (1998). Biomass density was estimated as the weight of biomass expressed as TS per unit volume of biofilm.

Evaluation of Mass Transfer Coefficients

A mass balance for the inert compound in each compartment of the flow cell can be made using the following equations, assuming that the biofilm thickness remained constant during the mass transfer measurements (i.e., the period when LiCl was introduced):

$$\frac{dC_I}{dt_e} = \frac{k_T \cdot A}{V_I} \cdot (C_{II} - C_I) \tag{1}$$

$$\frac{dC_{II}}{dt_e} = \frac{k_T \cdot A}{V_{II}} \cdot (C_I - C_{II}) \tag{1a}$$

By integrating Eqs. (1) and (1a) it is possible to obtain the change in lithium concentrations in both compartments (chambers) over time during the experiment:

$$C_I = C_* + (C_I^0 - C_*) \cdot \exp\left(-(t_e - t_e{}^0) \cdot k_T \cdot A \cdot \left(\frac{1}{V_I} + \frac{1}{V_{II}} \right) \right) \tag{2}$$

$$C_{II} = C_* - (C_* - C_{II}^0) \cdot \exp\left(-(t_e - t_e{}^0) \cdot k_T \cdot A \cdot \left(\frac{1}{V_I} + \frac{1}{V_{II}} \right) \right) \tag{3}$$

with the concentration at infinite time being:

$$C_* = \frac{C_I^0 \cdot V_I + C_{II}^0 \cdot V_{II}}{V_I + V_{II}} \tag{4}$$

C^0 and C are the lithium concentrations at $t_e = t_e{}^0$ and $t_e = t_e$, subscripts I and II indicate circuit I and II, respectively (e.g., V_I and V_{II} are the volumes of the two circuits), A is the mass transfer area, k_T the overall mass transfer coefficient (including the biofilm, the membrane and the external mass transfer resistances) and t_e is the time during which the lithium accumulates in circuit II. The symbol "t" indicates the age of the biofilm (see below, Figures 2 and 3).

The value of k_T was calculated by fitting equations (2) and (3) to the measured concentrations by non-

linear regression (Solver routine of MS-Excel).

The biofilm mass transfer coefficient at time t (time after the biofilm started to develop), k_b, can be calculated from the overall mass transfer coefficients at time t and at time t=0, respectively k_T and k_T^0 (the latter is the initial overall mass transfer coefficient when there is not biofilm, which includes the membrane and the external mass transfer resistances of the liquids in the two compartments):

$$\frac{1}{k_b} = \frac{1}{k_T} - \frac{1}{k_T^0} \tag{5}$$

Coefficient k_b represents the ratio between an effective average diffusivity and the average biofilm thickness.

Results and Discussion

No significant lithium adsorption on the biofilm was detected. This was verified by using a flow cell where the membrane was replaced with a non-porous flat plate and by letting the biofilm grow in chamber I under the same conditions as the biofilm grown on the membrane. After lithium was introduced, no permanent change in lithium concentration in the liquid was observed over time, meaning that lithium stayed mainly in the liquid and was not adsorbed by the biofilm.

Mass transfer measurements were performed during biofilm formation. Data were collected for 15 days at different stages of the biofilm development. All the experiments were undertaken in duplicate. Figure 2 shows one example of the several diffusion experiments conducted. From each of these experiments values for k_T and k_b could be estimated by using equations 2 to 5.

Figure 3 shows the changes in the biofilm mass transfer coefficient k_b over time for the tests performed at the three chosen upflow velocities. As expected, k_b decreased with time during biofilm formation due to the increase in thickness of the microbial layer. In all cases, biofilms reached steady-state after 10-14 days with a final value of k_b ($t \rightarrow \infty$) of about $1x10^{-6}$ m s^{-1} for Biofilms 1 and 2, and $1.55x10^{-6}$ m s^{-1} for Biofilm 3.

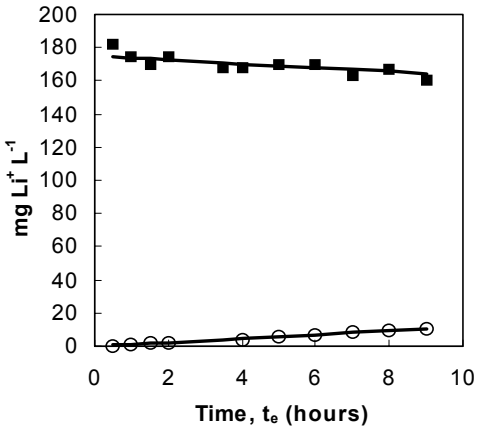

Figure 2. Example of experimental results of each side (biofilm of 2 days) v = 0.01 m s^{-1}. (■) Li^+ concentration in circuit I (C_I); (o) Li^+ concentration in circuit II (C_{II}); (-) modelled data (equations (2) and (3)).

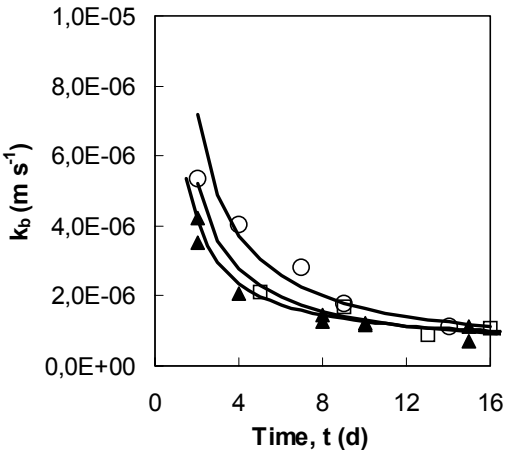

Figure 3. Biofilm mass transfer coefficient (□)Biofilm 1 (v = 0.004 m s^{-1}); (▲) Biofilm 2 (v = 0.010 m s^{-1}); (o)Biofilm 3 (v = 0.040 m s^{-1}).

Properties of the three biofilms are presented in Table 1. Biofilm 1 and 2 display identical characteristics, in spite of the liquid velocity being 2.5 times higher for Biofilm 2 than for Biofilm 1. On the other hand, the density and the k_b are greater in the case of Biofilm 3, which was formed under the highest velocity using a different carbon substrate (methanol). The total protein concentration increased with the liquid velocity, suggesting higher bacterial concentrations inside microbial films 2 and 3 than in Biofilm 1. The polysaccharide content was similar for Biofilms 1 and 2, but lower in the case of Biofilm 3. If erosion forces prevailed, bacteria would tend to reinforce their extracellular matrix by producing more polysaccharides per unit volume in Biofilm 3, but this did not seem to be the case here. The same conclusion is also suggested by the similar thicknesses of all three biofilms.

Table 1 Effect of upflow velocity on biofilm properties and lithium mass transfer (average values and standard deviations)

	Biofilm 1	Biofilm 2	Biofilm 3
Reynolds number (-)	77	190	732
Upflow velocity (m s^{-1})	0.004	0.010	0.040
Thickness (μm)	361±37	418±42	394±7
Density (kg dry biofilm m^{-3} wet biofilm)	30±5	29±1	49±7
Initial mass transfer coefficient in the membrane k_T^0 (m s^{-1})	$8.55x10^{-7}$±$7.07x10^{-9}$	$1.30x10^{-6}$±$1.27x10^{-7}$	$1.36x10^{-6}$±$1.10x10^{-7}$
Lithium mass transfer coefficient in biofilm, k_b (m s^{-1})	$9.1x10^{-7}$±$2.16x10^{-7}$	$9.27x10^{-7}$±$3.16x10^{-7}$	$1.55x10^{-6}$±$3.83x10^{-7}$
Total Protein (kg protein kg^{-1} dry biofilm)	0.22±0.05	0.5±0.01	0.59±0.11
Total Polysaccharides (kg polysaccharides kg^{-1} dry biofilm)	0.21±0.07	0.18±0.03	0.10±0.02

The relation between density and diffusivity is not straightforward. Although not very expressive, the higher k_b obtained with Biofilm 3 is attributed to the possible role of convective transport and to the different carbon source used. It should be noted that other authors (Casey *et al*., 2000) did not find also a clear correlation between biofilm density and the mass transfer coefficients. The results suggest a possible mechanism that controls maximum biofilm thickness, where the development of biofilm structure and thickness occurs until a certain critical overall mass transfer coefficient is reached.

Conclusions

A non reactive compound, LiCl, was used as a tracer to estimate biofilm mass transfer coefficients at different states of growth within denitrifying biofilms formed in a membrane flow cell under different flow conditions. Mass transfer measurements in conjunction with the determination of densities, thicknesses and protein and polysaccharide content produced relevant information on the characteristics of the biofilm and of the biofilm processes involved. Additionally, monitoring biofilm mass transfer coefficients over time is also an indirect method of estimating the development of the biofilm until it reaches the steady state.

Acknowledgments

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