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RESEARCH NOTE

MASS TRANSFER COEFFICIENTS WITHIN ANAEROBIC BIOFILMS: EFFECTS OF EXTERNAL LIQUID VELOCITY

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Abstract—This work concerns mass transport in anaerobic biofilms, formed under upflow liquid velocities similar to the prevailing conditions in anaerobic reactors used for wastewater treatment. During biofilm formation under liquid velocities of 1.5 and 13.2 m/h, internal mass transfer coefficients were routinely measured. Mass transfer coefficients attained pseudo steady-state values between 2–4×10^{-3} m/h, no dependence being observed between bulk flow and internal mass transport rates. However, a transient variation in the liquid velocity from 1.5 up to 13.2 m/h, imposed after the biofilm had reached the steady-state, increased the internal mass transport by 20% on average. This result suggests that periodic changes in the bulk fluid velocity can be used as a tool to increase the transport of soluble substrates inside already formed biofilms, although the effect seems to be limited. © 1999 Elsevier Science Ltd. All rights reserved

Key words—anaerobic biofilms, internal mass transfer, hydrodynamics, low strength wastewaters

NOMENCLATURE

- V volume of the diffusion side, compartment II (L^3)
- C_1 lithium concentration in the bulk liquid of compartment I (ML^{-3})
- C_2 lithium concentration in the bulk liquid of compartment II (ML^{-3})
- A area of mass transfer (L^2)
- j mass transfer flux ($ML^{-1}T^{-1}$)
- k mass transfer coefficient (LT^{-1})
- k_b mass transfer coefficient within the biofilm (internal) (LT^{-1})
- k_{ml} mass transfer coefficient of the liquid film (external) plus membrane (LT^{-1})
- k_t mass transfer coefficient of the biofilm plus liquid film and membrane (LT^{-1})

INTRODUCTION

Anaerobic processes represent an appropriate technology for the treatment of many industrial effluents. Biomass accumulation within anaerobic reactors is provided by the formation of microbial aggregates by adhesion to a support material or by a self-aggregation process. Mass transfer in those biological structures may be described by mass transfer coefficients or by effective diffusivities, both encompassing all solute, solvent and local geometry interactions. Molecular diffusion has been considered the major transport mechanism and diffusivities lower than the correspondent value in water are reported in experiments carried out in anaerobic biofilms under steady-state conditions (Nilsson and Karlsson, 1989; Ozturk *et al.*, 1989; Kitsos *et al.*, 1992). However, mass transport by convective flow of bulk liquid within porous structures, previously theorised by Nir and Pisman (1977), was recently observed in aerobic biofilms by de Beer *et al.* (1994), who identified internal flows using a confocal laser microscope technique. These findings support the need for more research on transport phenomena in anaerobic biofilms. Indeed, mass transfer limitations are referred to in methanogenic processes (Kitsos *et al.*, 1992; Brito and Melo, 1997) and the effect of external flow as an active agent in the internal transport process in anaerobic systems has been raised, but not experimentally verified and quantified (Alphenaar *et al.*, 1993; Kato *et al.*, 1994).

The present experimental work was carried out to study the relationship between internal mass transport in methanogenic biofilms and bulk fluid velocities similar to the ones used in full-scale operation of anaerobic reactors. To accomplish this objective, two different experiments were performed:

1. measurement of internal mass transfer coefficients during the process of biofilm formation under two different bulk fluid velocities;
2. evaluation of the sensitivity of the internal mass transfer coefficient in fully established biofilms to a transient shift in the external bulk liquid velocity.

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The experiments were carried out in a membrane flow cell and were based on the measurement of the transport of lithium through biofilms adhered to the porous membrane.

MATERIAL AND METHODS

The inoculum of suspended biomass was provided by the disaggregation of anaerobic granules collected in a full scale UASB reactor, from Roermond B.V., The Netherlands.

The substrate composition simulated a low strength wastewater, being a volatile fatty acid (VFA) mixture of acetic (50%), propionic (25%) and butyric acid (25%). The average concentration in the flow cell remained between 100–200 mg/l of volatile fatty acids. The substrate was supplemented with a macro- and a micro-nutrients solution, as described elsewhere (Brito and Melo, 1997).

The experimental set-up of the mass transfer flow cell is depicted in Fig. 1.

The flow cell was made of acrylic glass and had two separate compartments of semi-circular cross section, with a radius of 0.025 m. These two compartments were interconnected by a 0.03×0.107 m aperture in the adjacent walls where, inbetween, a porous membrane (0.22 μm) was fixed. To prevent entry effects, the distance between the flow cell inlet and the membrane was 1.14 m. The biofilm was formed on the membrane side where the inoculum was recirculated (compartment I). Both compartments were opened to the atmosphere to enable the gas release. The top of compartment II was refrigerated at 6°C, in order to prevent water evaporation and induced liquid flow from compartment I. The effluent stream from compartment I was discharged by another pump to avoid air entrainment within the discharge pipe to reservoir 1. The whole system was thermoregulated by a water jacket, kept at 28 ± 1°C.

The surface of the biofilms was observed off line using a video monitor connected to a microscope video-camera, Mitsubichi Microwatcher VS-30H, equipped with a lens possessing a magnification up to $200 \times$. Biofilm thickness was measured with a digital micrometer, Mitutoyo, having a needle fixed on its moving arm. Biofilm samples were examined by Scanning Electron Microscopy (SEM). Biomass was carefully scraped from the membrane after the measurement of biofilm thickness and solids were determined as indicated in Standard Methods (1989). Volatile fatty acids concentration was measured with an HPLC. The lithium ion source was lithium chloride. Lithium was measured, as Li^+, both by a flame photometer and by conductivity with an on-line cell probe. Conductivity measurements were continuously registered.

The mass transfer measurement approach was based on the following mass balance:

$$V\frac{dC_2}{dt'} = Aj \tag{1}$$

or

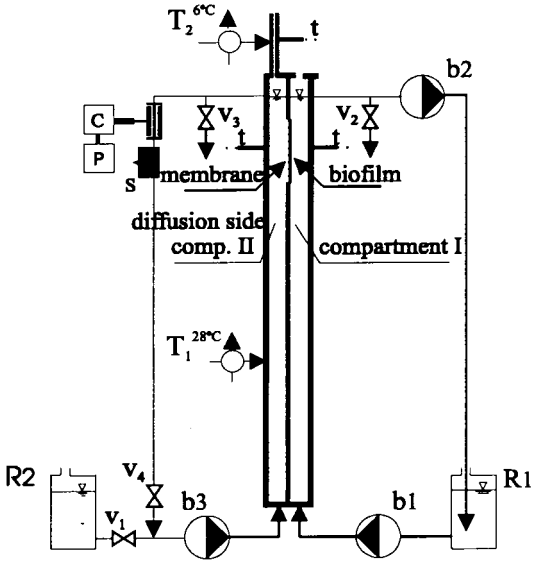
$$V\frac{dC_2}{dt'} = Ak(C_1 - C_2) \tag{2}$$

where V refers to the volume of the diffusion side, compartment II, (m^3), C_1 and C_2 are the lithium concentrations in the compartments I and II (mg/l), A is the area of mass transfer (m^2), j is the mass transfer flux (mg/m^2h), k (m/h) is the overall mass transfer coefficient and t' is the time measured during each lithium mass transfer determination. Considering C_1 constant, separating variables and integrating with the appropriate boundary conditions ($C = 0$, $t = 0$), the following equation is obtained, the mass transfer value being extracted by a least squares method:

$$\ln\frac{(C_1 - C_{2(t')})}{(C_1 - C_{2(t'=0)})} = -k\frac{A}{V}t' \tag{3}$$

Mass transfer coefficients across the different media, i.e. liquid film, biofilm and membrane are combined in an overall mass transfer coefficient, k_t. If k_b is the internal (biofilm) mass transfer coefficient, its reciprocal is the difference between the overall mass transfer resistances evaluated with and without biofilm, respectively, neglecting partition effects:

$$\frac{1}{k_b} = \frac{1}{k_t} - \frac{1}{k_{ml}} \tag{4}$$



Caption:
b1 - influent pump
b2 - effluent pump
b3 - recirculation pump
T_1,T_2 - thermorecirculation pumps
(water-jackets)
t - thermocouples connected to thermometer
C - conductivity meter with on-line cell
P - printer
S - sampling port
v_1, v_2 , v_3, v_4- valves
R1 - reservoir 1 *(nutrients and tracer injection)*
R2 - reservoir 2

Fig. 1. Mass transfer flow cell.

where $k_{(t=t)} = k_t$ is the overall mass transfer coefficient and $k_{(t=0)} = k_{ml}$ is the external mass transfer coefficient plus the membrane mass transfer coefficient (without the biofilm on the membrane).

Instead of making assumptions about biofilm kinetics or inactivating the biomass, lithium chloride was used as a non-reactive tracer that diffuses through the biofilm (Kitsos *et al*., 1992). Lithium ion concentration was kept lower than 500 mg/l to avoid possible inhibition effects (Anderson *et al*., 1991). Concentration in the diffusion side was typically between 1–10 mg/l of Li^+.

Two different types of experiments were performed, as follows:

- Type 1. Biofilms were formed under two distinct bulk upflow liquid velocities (1.5 and 13.2 m/h) and the overall lithium mass transfer coefficients were regularly measured along the 15–20 days of each experiment. The determination of each lithium mass transfer coefficient required at least 6 samples of 2 ml, periodically extracted from the diffusion compartment during approximately 3 h and immediately analysed. In this experiment, the solids concentration of the inoculum in compartment I and reservoir 1 was approximately 10 kg volatile solids/m^3, and was continuously recycled. Lithium concentration in compartment I–R_1 was monitored periodically and maintained approximately constant by adding some lithium chloride in R_1 when necessary.
- Type 2. Biofilms were formed during a period of approximately 10 days under given upflow liquid velocities (0.9 m/h, 1.5 m/h, 7.1 m/h, 13.2 m/h and 13.7 m/h). Based on previous experience, this period was considered to be sufficient to achieve a pseudo steady-state biofilm. Then, lithium chloride was added in R_1, being immediately dissolved and homogenised into the stirred liquid. Afterwards, each pair of upflow liquid velocities was sequentially imposed. The test using a liquid velocity for biofilm formation at 0.9 m/h, one of the tests using 1.5 m/h, the test using 7.1 m/h and one of the tests using 13.2 m/h were first carried out at the low velocity (1.5 m/h). The remaining tests were first performed applying the higher liquid velocity, that is, 13.2 m/h and thereafter the low velocity. In these experiments, conductivity was on-line registered in the diffusion compartment. Previously, distilled water was circulated in both sides of the cell during 3–4 h. The correlation coefficient between the conductivity increase and lithium concentration in the diffusion side was 0.999. Each pair of mass transfer coefficients obtained under a given velocity was assessed after the formation of a new biofilm and biomass in suspension was forced to wash out of the system after 4 d.

During each mass transfer determination, compartments I and II worked in a closed loop (valves v1, v2, v3 were closed and v4 open). When it was necessary to wash the system, valves v1, v2 and v3 were open and v4 was closed, while R1 and R2 were filled with distilled water.

The upflow liquid velocities of 1.5 and 13.2 m/h were selected because they are in the range most anaerobic reactors are designed for. Before each system inoculation, blank assays (i.e. using the described substrate composition but without biofilm) were always carried out applying flow conditions identical to the ones used in the biofilm test. Such assays were used to calculate k_{ml}, that is, the membrane mass transfer coefficient plus the liquid film mass transfer coefficient (external resistance) in both compartments. In the beginning and at the end of each experiment, lithium concentration and conductivity were also measured in the reservoir side and the mass balance indicated that adsorption of lithium was not significant.

RESULTS

Mass transfer within biofilms formed under different upflow liquid velocities

Figs 2 and 3 present the lithium internal mass transfer coefficients obtained during biofilm formation (experiments of Type 1, described in the section 'Material and methods'). Results are presented for the two different velocities tested, 1.5 and 13.2 m/h. The Reynolds numbers calculated with these velocities, taking the cell hydraulic diameter as the characteristic length, were 25 and 222, representing laminar flow in the mass transfer cell. Since the laminar boundary layer is fully developed, the external mass transfer coefficient will not depend on the liquid velocity. Theoretical shear stresses in the flow cell wall were 0.6×10^{-4} and 4.8×10^{-4} N/m^2, assuming one-phase laminar flow.

It can be observed that in both conditions the biofilm mass transfer coefficients decline from their initial value, reaching a more or less stable value (pseudo steady-state) in near 10 days or less. Values of the internal mass transfer coefficients in the final (pseudo steady state) period of biofilm formation range generally between $2–4 \times 10^{-3}$ m/h, but are not dependent on the bulk liquid velocity.

The average thickness of each biofilm ranged from near 250 μm up to 350 μm, showing an irregular surface. The solids content of the different biofilms were similar, between 32–36 kg of total solids/m^3. Lithium ion diffusivities may be obtained by multiplying the mass transfer coefficient by biofilm thickness. Considering an average thickness of 300 μm, an average diffusivity of 0.9×10^{-6} m^2/h is obtained in such steady biofilms, representing 33% of the diffusion coefficient of ion lithium in water, which is 2.7×10^{-6} m^2/h (Kitsos *et al*., 1992). SEM micrographs revealed filamentous bacteria morphologically resembling *Methanothrix* spp., an observation in accordance with the type of inoculum used (Hulshoff Pol *et al*., 1988).

Mass transfer within biofilms: response to a transient shift in bulk fluid velocity

Table 1 presents the results obtained in the experiments performed with pseudo steady-state biofilms formed under different bulk fluid velocities (Type 2 experiments).

The results were tested against statistical methods in order to assess whether the differences between the mass transfer coefficients obtained by changing the fluid velocities could be considered significant. Because the dependent variables are two related samples, a paired comparisons test using Student's *t*-test distribution based on differences was performed (Daniel, 1987). The conclusion points to a significant statistic beyond the 1% level. The 'null hypothesis' assumed that the fluid velocity had no impact on the observed mass transfer differences and was rejected because the *P*-value was −8.36

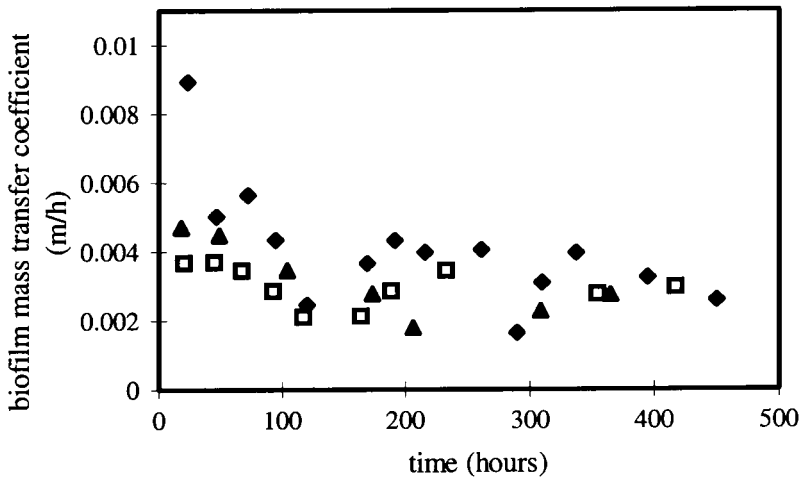


Fig. 2. Internal mass transfer coefficients during biofilm formation under a liquid velocity of 1.5 m/h (data from 3 similar experiments of Type 1).

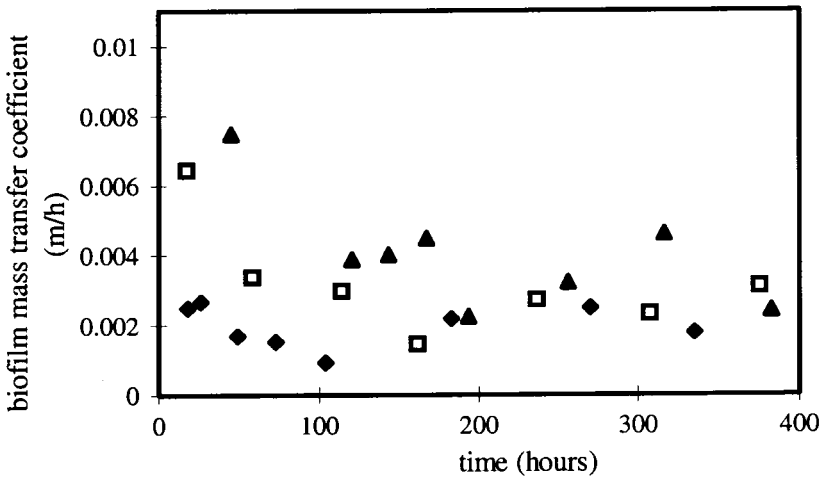


Fig. 3. Internal mass transfer coefficients during biofilm formation under a liquid velocity of 13.2 m/h (data from 3 similar experiments of Type 1).

while the critical t value of the Student's t-test for a significant level of 0.01 is -3.143. The binomial test, which is a non-parametric statistics appropriate when a small number of paired samples is available, also indicated a significative trend at a confidence level of 98%. The conclusion is that the differences between the paired values are significant, that is, the change imposed on the external liquid velocity did affect mass transfer rates inside the biofilm.

DISCUSSION

As can be seen in Figs 2 and 3, despite the fact that two different liquid velocities were applied during biofilm formation, the internal mass transfer coefficients at pseudo steady-state conditions were similar. The fluctuations of k_b values may be attributed to the irregularity of the biofilm surface, since it affects the true value of the biofilm surface area (A, which was considered constant when using Eq. (4)). Furthermore, the average thickness of these biofilms did not change significantly with the fluid velocity. Consequently, when comparing two biofilms formed under different liquid velocities, in laminar regime, it can be assumed that the diffusivities within the biofilms were similar because the mass transfer coefficient k_b is seen as the ratio of the effective diffusivity to the biofilm thickness. Mass transfer studies during biofilm formation in heat exchangers (Vieira *et al.*, 1993), under turbulent liquid flows (much higher velocities, ranging from 1.2×10^3 m/h to 2×10^3 m/h, and thus higher shear stresses) showed that the final values of the mass transfer coefficients were also similar, regard-

Table 1. External liquid velocity effects on internal mass transfer coefficient (Type 2 experiments)

Liquid velocity during biofilm formation (m/h)	Mass transfer coefficient, k_{b1} (m/h) (liquid velocity: 1.5 m/h)	Mass transfer coefficient, k_{b2} (m/h) (liquid velocity: 13.2 m/h)	$(k_{b2}-k_{b1})/k_{b1}$ (%)
0.9	0.0018	0.0023	28
1.5	0.0019	0.0022	16
1.5	0.0028	0.0034	21
7.1	0.0026	0.0030	15
13.2	0.0021	0.0024	14
13.2	0.0028	0.0035	25
13.7	0.0014	0.0018	21

less of the liquid velocities under which the biofilm was formed. However, contrary to the present work, the flow velocity had a pronounced effect on the biofilm thickness: higher velocities lead to much lower thicknesses and to a higher degree of compactness of the biofilm. In the present study, at low substrate concentrations and where fully laminar flow prevails in all experiments, the compactness of the biofilms was not affected by the external hydrodynamics, as confirmed by the similar values of the solids content in all biofilms (32–36 kg total solids/m^3).

The absence of a significant effect of liquid velocity, when comparing Figs 2 and 3, should be expected since it is known that in laminar flow there are no 'turbulent bursts' and 'downsweeps' acting on the biofilm surface, which lead to higher detachment rates and mass transport (Cleaver and Yates, 1973; Cleaver and Yates, 1975). In spite of that, in the case of highly loaded reactors, where gas evolution may be important, a significant turbulence level can develop near the biofilm surface, thus affecting mass transport (Huisman *et al*., 1990) and detachment mechanisms, even if the liquid Reynolds number falls well within the laminar regime.

However, a different phenomenon was observed when velocity shifts were imposed upon biofilms previously formed under different hydrodynamic conditions. In fact, the statistical analysis of the results displayed in Table 1 shows that the application of transient fluid velocities to an already formed biofilm has an impact on the internal mass transfer rates within that biofilm. This effect could be driven by the ability of external flow-induced pressure gradients to transport liquid into the biofilm matrix. Convective mass transfer mechanisms were previously reported in aerobic biofilms (Siegrist and Gujer, 1985; de Beer *et al*., 1994). Moreover, it is possible that the increase of the liquid pressure could reduce the gas hold-up inside the biofilm, the removal of the gas being compensated by a liquid flow into the biofilm, an effect referred to in tower anaerobic reactors (Van den Heuvel *et al*., 1992). These positive effects may favour the use of pulsed reactors in wastewater treatment (Stadlbauer *et al*., 1992; Brito *et al*., 1997). In the experiments of Type 2, the biofilms were not given enough time to adjust their structure to the changes in the fluid velocity, since the study was performed in very few hours; this may explain why different external hydrodynamic conditions could affect (although slightly) the internal mass transport rates. The impact of liquid changes will certainly depend on the biofilm structure and it should be stressed that the relationship between bulk velocity and internal convection was rather limited in the present work: there was an 8.8 times increase in the liquid flow rate but the recorded increase in the biofilm mass transfer coefficient was on average 1.2 times. Convective effects could also be present in the experiment where the liquid velocity was kept constant (Type 1) but such effects could be masked by the ability of the biofilm to adjust itself to different environmental conditions during its development, resulting in similar final resistances to mass transfer in all experiments.

CONCLUSIONS

Anaerobic biofilms were formed in a flow cell where internal mass transfer coefficients were measured. The following experimental evidence was provided in this work:

1. The measurements of the internal mass transfer coefficients during the process of biofilm formation do not point to a clear effect of the bulk liquid velocity on the internal transport properties provided that the velocity is kept constant during the whole experiment: mass transfer coefficients attained similar levels at pseudo steady-state conditions under laminar regime.
2. Internal mass transfer coefficients in each pseudo-steady state biofilm were affected by a shift in the bulk fluid velocity at the end of the experiment. An additional internal mass transport flux was measured when the bulk fluid velocity was increased 8.8 times, although the effect was rather small, leading only to a 20% average increase in the internal mass transfer coefficient.

Therefore, the results suggest that by imposing transient changes of the liquid flow in laminar regimen in contact with fully established biofilms, one may induce changes in the internal mass transfer coefficients, probably due to additional convection

effects. On the other hand, if the liquid velocity is kept constant during biofilm formation, the steady states values of the internal mass transfer coefficients are similar in all biofilms, regardless of the hydrodynamics conditions, at least in laminar regimen. Consequently, more information about biofilm properties is much needed. Direct methods like the one presented here may then be useful to obtain biofilm mass transfer coefficients for the purpose of reactor's modelling and design.

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