

Measurement of Local Mass Transfer Coefficient in Biofilms

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Local mass transfer rates for an electrochemically formed microsink in an aerobic biofilm was measured by a mobile microelectrode using limiting current technique. Mass transfer coefficients varied both horizontally and vertically in the biofilm. The results implied the existence of an irregular biofilm structure consisting of microbial cell clusters surrounded by tortuous water channels. An unexpected increase of the local mass transfer coefficient just above the biofilm surface suggested the existence of local flow instability in this region. As expected, the influence of bulk flow velocity on the local mass transfer rate decreased with increasing depth into the biofilm. Mass transfer coefficients fluctuated significantly inside microbial cell clusters, suggesting the existence of internal channels through which liquid could flow. A new conceptual model of biofilm microbial cluster structure is proposed to account for such biofilm microstructure irregularities. © 1995 John Wiley & Sons, Inc. Key words: biofilm • mass transfer coefficient • microelectrode

INTRODUCTION

Steady-state biofilm models^{5,17,18} assume that the internal mass transfer (inside the biofilm) is diffusion controlled and that the external mass transfer (outside the biofilm) is dominated by convection. Consequently, it is believed that the molecular diffusivity is the only parameter needed to describe mass transfer rates inside biofilms. Mass transfer rate from the bulk liquid to the biofilm surface is commonly approximated by assuming that the mass boundary layer above biofilm surface is uniform and stagnant.

This simple model of mass transport in biofilms has been challenged by recent reports^{1–3,12,21} that reveal that structures of aerobic biofilms are not uniform but consist of microbial cell clusters separated by interstitial voids. According to this concept, mass transport in the interstitial voids is mainly facilitated by convective flow and mass transfer inside the microbial clusters is entirely due to molecular diffusion. Therefore, the mechanism of local mass transport is determined by the structural heterogeneity of biofilms.

Consequently, mass transfer rates are not the same at different locations in a biofilm due to variable porous structures and reactivities that vary in space. Rather, mass transfer rates depend on complex interactions among local bio-

film activity, structure, and hydrodynamics. The situation is further complicated because biofilms are viscoelastic and microscale hydrodynamics near such surfaces has not been characterized analytically. Because there is no adequate mathematical model describing mass transfer in a growing three-dimensional irregular structure, it is necessary to study the mass transfer rate inside heterogeneous biofilms experimentally.

A few attempts have been made to describe the relations between biofilm structure and hydrodynamics. Lewandowski et al.¹³ described the liquid flow in a biofilm reactor using nuclear magnetic resonance imaging (NMRI) and the liquid movement in the space occupied by the biofilm. De Beer et al.¹ and Stoodley et al.²¹ measured liquid flow velocities in biofilm voids by using particle image velocimetry (PIV), which traces the horizontal movement of fluorescent beads in the voids of biofilms using the confocal scanning laser microscope (CSLM). Zhang and Bishop²⁴ described the porosity and pore structure of biofilms. Although these studies quantify factors influencing mass transport rate, none of them provides the means to actually measure it.

This article describes measurements of local mass transfer coefficients in biofilms using a modified limiting current technique (LCT).^{6,14,23} LCT uses a particular electrochemical reaction which proceeds at the maximum possible rate on an electrode surface. Under mass transfer limiting conditions, the magnitude of electrical current measured in the external circuit depends only on the mass transfer rate and is directly proportional to the mass transfer coefficient to the electrode. This well-known technique is frequently used to study mass transfer rates^{4,9–11,19} to flat surfaces. Electrodes used are flush mounted to the reactor's wall, and range in size from several hundred micrometers to a few centimeters.

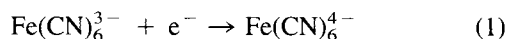
Our application of LCT is different from the conventional method in two aspects: (1) the microelectrode used here was mobile and could be positioned at any location in biofilms; and (2) the size of the electrode was reduced to a few micrometers to prevent damaging the biofilm structure and disturbing the local hydrodynamics.

The tip of the microelectrode formed a microsink for the electroactive species purposely added to the reactor. The measured limiting current was directly proportional to the local mass transfer rate to the microelectrode. The magnitude of the limiting current was dependent on combinations

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of all factors influencing local mass transport rate, e.g., local hydrodynamics and the local structure of the biofilms. Consequently, local mass transfer coefficients calculated from the measured limiting current to the microelectrode is related to the local effective diffusivity which is a function of local hydrodynamics and local biofilm structure.

A typical redox system, ferri-ferrocyanide, was selected and used in this study. With proper experimental control, the electrochemical reduction of $\text{Fe}(\text{CN})_6^{3-}$ occurred on the surface of the microelectrode



By increasing the polarization potential, the current will increase until the concentration of $\text{Fe}(\text{CN})_6^{3-}$ at the electrode surface reaches zero ($C_s = 0$). The current corresponding to a zero surface concentration of the reacting species is called "the limiting current." The flux of ferri-cyanide ion, J , to the surface of the microelectrode with sensing area A is related to the limiting current, I

$$J = \frac{I}{nAF} \quad (2)$$

where F is Faraday's constant and n is the number of mole of electrons transferred in the reaction. The flux is also related to the mass transfer coefficient, k ,

$$J = k(C_o - C_s) \quad (3)$$

where C_o is the concentration of the reacting species in the bulk, and C_s the concentration at the surface of the microelectrode. The relationship between mass transfer coefficient and limiting current can be found by combining Eqs. (2) and (3) for $C_s = 0$

$$k = \frac{I}{nAFC_o}$$

MATERIALS AND METHODS

The Biofilm Reactor

An open channel biofilm reactor used in this research was made of transparent polycarbonate. It was 2 cm deep, 4 cm wide, and 75 cm long. The biofilm nutrient solution consisted of KH_2PO_4 (2.2 mM), K_2HPO_4 (4.8 mM), $(\text{NH}_4)\text{SO}_4$ (0.25 mM), MgSO_4 (0.04 mM), and yeast extract (0.1 g/L) and was recycled through the reactor at a rate of 10 mL/s by a magnet drive vane pump (Cole-Parmer, Chicago, IL). Oxygen was supplied by preaerating the nutrient solution. The operating volume of the recycle loop including the reactor was 450 mL. After inoculation, the reactor was operated batchwise for 12 h. The inoculum consisted of organisms: *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, and *Klebsiella pneumoniae*. Use of this defined mixed population to form biofilms has been described in more detail elsewhere.²¹

After 12 h of batch operation, the reactor was switched to

a continuous flow mode. Culture medium was added by a peristaltic pump (Masterflex; Cole-Parmer, Niles, IL) at a rate of 15 mL/min to achieve a hydraulic residence time of 30 min. The same peristaltic pump was used to maintain the effluent flow at the same flow rate. The biofilm was allowed to grow for 4 to 7 days before LCT measurements.

Just before LCT measurement, the flow of nutrient in the reactor was stopped, the nutrient solution in the reactor was slowly drained, keeping the biofilm moist at all times, and replaced with the electrolyte solution formulated for the LCT measurements. The reactor was then connected to a gravitational flow system designed for LCT measurement.

Electrodes and Electrolyte Solution

Microelectrodes used for LCT measurements were made of 100 μm platinum wire, 99.99% Pt (product of Engelhard, Carteret, NJ), with the tips etched electrochemically in 2 M KCN solution to a diameter of less than 1 μm . The wire was then rinsed with double-distilled water and carefully inserted into a glass capillary. Location of the wire's tip in the capillary was observed under a microscope. The glass capillary was positioned in a microelectrode puller (Stoelting Co., Wooddale, IL), and the tip of the wire was positioned 1 to 1.5 cm higher than a Ni-Cr heating coil.

Heat was gradually increased until the glass around the platinum wire melted and adhered to the wire while the entire capillary dropped down and cooled in the air. The tip of the wire was then exposed by grinding on a rotating diamond wheel (Model EG-4, Narishige Co., Tokyo, Japan). The surface of the microelectrode was cleaned in a sonication bath first with deionized water, then with acetone. The tip diameter was measured microscopically and the surface area of the electrode calculated. Typically, the tip diameters were between 3 μm and 10 μm . An electrical connection to the microelectrode was made by inserting a small piece of solder and a bare copper wire into the capillary and melting the solder in a stream of hot air to form a metallic droplet connecting the platinum and copper wires.

The counter/reference electrode was a commercial calomel electrode (Model 13-620-51, Fisher Scientific, Pittsburgh, PA). A Hewlett Packard 4140B multimeter was used as voltage source and picoammeter. The microelectrode was polarized cathodically to -0.8 V against the reference electrode that was placed next to it within the reactor in the downstream direction. Voltammetry tests were performed for each microelectrode to check their performance. Voltage between the microelectrode and the reference electrode was scanned from 0 to -1.2 V. Microelectrodes showing limiting current in the voltage range between -0.6 to -0.9 V were considered usable.

The reaction/diffusion solution consisted of 0.5 M of KCl and 25 mM of $\text{K}_3\text{Fe}(\text{CN})_6$. KCl was used as supporting electrolyte to suppress the contribution of electromigration to the overall mass transfer rate to microelectrode. Viscosity of this solution, μ , as measured with a falling ball viscom-

eter (Model GV-2100, Gilmont Instruments, Barrington, IL) was 0.9888 cP. Density of the solution, ρ , was 1.017 g/mL. Actual concentrations of $\text{Fe}(\text{CN})_6^{3-}$ were determined by iodimetric titration²² before each measurement.

The reaction/diffusion solution was recycled through the biofilm reactor using a gravitational flow system to ensure flow rates were constant and without pulsation. Flow rates were adjusted by a control valve and continuously monitored by a flowmeter (Model GF-1500, Gilmont Instruments). The recirculating electrolyte was filtered through a quartz wool plug to remove any large aggregates of cells. Before measurement, electrolyte solution was allowed to flow through the biofilm reactor for 30 min to allow complete distributing throughout the reactor.

Biofilm Monitoring

The biofilm reactor was positioned on the stage of an inverted microscope (Model CK-2, Olympus, Japan). Biofilm structure was observed through the transparent bottom of the reactor. During the LCT measurement, microphotographs of biofilm were taken hourly to check for structural integrity of the biofilms and samples of biofilm were taken for R2A agar plate count to determine the viability of biofilm at times 0, 2, 4, and 22 h. For the plate count, the biofilm from a known surface area was mechanically removed and transferred to a known volume of buffer solution. The sample was homogenized and the solution was spread over the agar plate. The colony-forming units were counted after overnight cultivation.

Measuring Vertical Profiles of Mass Transfer Coefficient

A microelectrode was mounted on a micromanipulator (Model M3301L, World Precision Instruments, New Haven, CT) that was equipped with a stepper motor (Model 18503, Oriel, Stratford, CT) and manipulated by a computer controller (Model 20010, Oriel). The microelectrode was introduced from the top of the reactor perpendicular to the bottom of the biofilm. To ensure that the viscous flow profile at the location of the measurement was fully developed, the distance from the chosen measurement location to the entrance of reactor was always larger than the hydrodynamic entrance length as estimated according to Schlichting's equation,⁶ $L = 0.01 \times 4 \times R_H \times Re$, where R_H is the hydraulic radius (in centimeters) and Re is the Reynolds number. We calculated the hydraulic radius as the ratio of the wetted cross-section to the wetted perimeter.

Location of the electrode tip in the biofilm was monitored microscopically. To measure vertical profiles of the mass transfer coefficient, the microelectrode was slowly moved down from the bulk liquid and into biofilm at preset increments by the computer-controlled micromanipulator. Limiting current was measured at various vertical positions and stored by the data acquisition system. The minimum dis-

tance between two adjacent positions, for microelectrodes with a tip diameter of 10 μm , was greater than 20 μm .

To establish the vertical z -coordinate, the position of the reactor's bottom plate (the substratum for the biofilm) was set as $z = 0$. The vertical position of the microelectrode, z , was then measured as the distance from tip of the microelectrode to the bottom of the reactor. The lowest limiting current was always measured at $z = 0$.

Custom software was used to control microelectrode movement and collect limiting current data. The vertical profile of limiting current was displayed on a computer monitor in real time. At each vertical position, the data acquisition system (Model CIO-DAS08PGL, ComputerBoards, Inc., Mansfield, MA) collected 22 separate readings at a sampling frequency of 1 kHz. The highest and the lowest values were rejected and the remaining 20 measurements were averaged. Standard deviation was calculated for each set of 20 data readings and compared with a preset standard deviation defining the desired precision of the measurement. If the measured standard deviation was smaller than the preset one, the measurement was accepted, the data stored, the result displayed on the screen, and the microelectrode moved to the next vertical position. If the calculated standard deviation was higher than the preset one, the measurement was repeated. A compromise between precision and practicality had to be accepted to complete the measurements in a reasonable time. A relative standard deviation of 0.05 was used as an arbitrary acceptable level for all measurements.

Limiting current profiles were measured through both the biofilm clusters and the interstitial voids. Vertical position of the top of the cluster was estimated from the mass transfer coefficient profile: the point at which the mass transfer coefficient showed the first dramatic decrease. Estimations of the cluster locations were confirmed by microscopic observation.

Measuring Horizontal Distributions of the Mass Transfer Coefficient

The biofilm reactor was positioned on an X-Y micropositioner stage (Model CTC-462-2S, Micro Kinetics, Laguna Hills, CA) with the microelectrode attached to a linear micropositioner (Model CTC-322-20, Micro Kinetics) and positioned above the reactor. Figure 1 is the schematic diagram of the experimental setup. The micropositioners were computer controlled through a controller (Model CTC-283-3, Micro Kinetics) with a positioning precision of 0.1 μm . Custom software was used to simultaneously control the stage movement, microelectrode movement, data acquisition, and distribution display in real time. The software allowed sampling a horizontal grid over a specific area. The usual grid setting was a 10×10 matrix, with a step size of 25 μm in both X and Y horizontal directions. The same data acquisition protocol as used in vertical profile measurement was applied.

The microelectrode was manipulated manually to the first

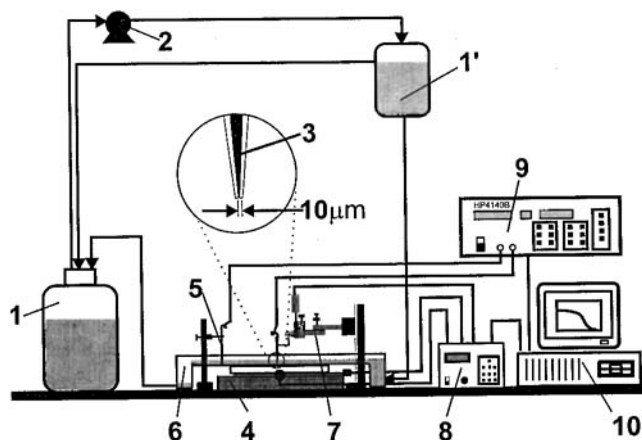


Figure 1. The schematic diagram of LCT setup for horizontal distribution measurement of local mass transfer coefficient: 1 and 1'—electrolyte reservoir; 2—pump; 3—microelectrode; 4—X-Y micropositioner stage; 5—counter/reference electrode; 6—open channel reactor; 7—linear micropositioner; 8—controller; 9—multimeter; 10—computer.

grid point at a predetermined position in the biofilm. After the limiting current was measured and the data was accepted, the linear micropositioner moved the microelectrode out of the biofilm into bulk liquid, the motorized X-Y stage moved the reactor to the next grid point, the microelectrode was lowered to the predetermined position, and the limiting current was measured after a 40-ms delay. The procedure was repeated for the entire grid. The horizontal distribution of limiting current was displayed on the computer's monitor in real time. The real-time result display permitted, on many occasions, early detection of experimental problems, such as data acquisition malfunction, that could have otherwise remained unnoticed until the measurement was completed.

To evaluate the influence of convective flow on mass transfer rate, the horizontal distributions of mass transfer coefficient at the same vertical position were measured at various bulk flow velocities.

RESULTS

Biofilm Structure and Viability

Microscopic observation supported by time-lapse photography revealed that the biofilm structure remained intact for more than 20 h under the continuous recycle flow of the reaction/diffusion electrolyte solution. No visible detachment or sloughing was recorded during the mass transfer measurement. Although it was assumed that the biofilm was inactive under the conditions of the mass transfer experiments (because of the osmotic shock due to the high ionic strength and the presence of the ferri-ferrocyanide) the results of plate counts showed that the number of viable cells in the biofilm remained relatively constant over first 4 h from the onset of electrolyte recycling (Table I).

Table I. Viable cell counts of the biofilm under LCT measurement condition.

	Sampling time			
	0	2 h	4 h	22 h
Viable cell count (CFU/cm ²)	4.8×10^7	3.6×10^8	1.2×10^8	1.2×10^5

CFU = colony forming units.

Vertical Profiles of Mass Transfer Coefficient

Two typical vertical profiles of mass transfer coefficient are illustrated in Figure 2. Curve (a) was measured in a water-filled interstitial void and is similar to that measured in a clean reactor (results not shown). This profile shows that the local mass transfer coefficient in the void decreased gradually toward the bottom of the reactor. Curve (b) was measured through a 170-μm-thick biofilm cluster. For the vertical position above $z = 700 \mu\text{m}$, the local mass transfer coefficient was constant. Between $z = 400$ and $700 \mu\text{m}$, a decrease of the local mass transfer coefficient was observed. Just above the top of the cluster, between $z = 200$ and $400 \mu\text{m}$, the local mass transfer coefficient increased slightly. Inside the biofilm cluster, the local mass transfer coefficient fluctuated significantly.

In a separate experiment, two profiles were taken at two horizontal locations, 200 μm apart, in a 450-μm-thick section of biofilm (Fig. 3). Profile (a) demonstrates a similar randomly fluctuating mass transfer coefficient as observed in Figure 2. Profile (b) shows a rather smooth decrease in the local mass transfer coefficient.

Horizontal Distribution of Mass Transfer Coefficient

Measurements of the local mass transfer coefficient at a horizontal plane, at various vertical positions, were con-

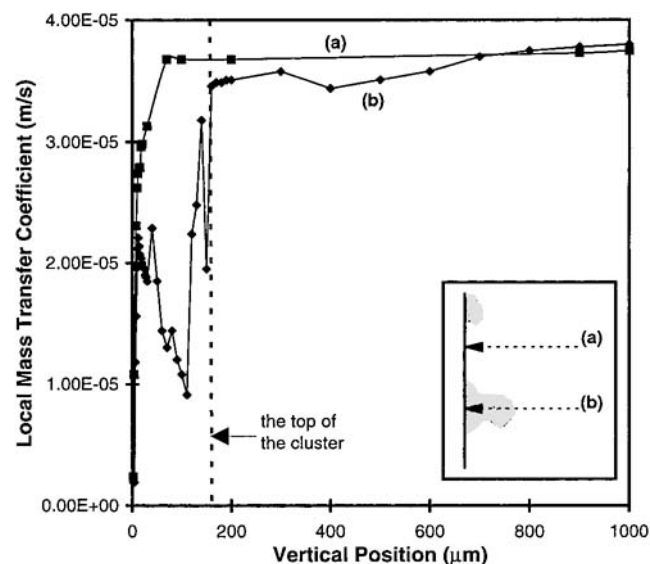


Figure 2. Vertical profiles of local mass transfer coefficient measured in a void (a) and a cluster (b). The insert is the schematic diagram that shows locations of measurement. The shaded area represents biofilm clusters.

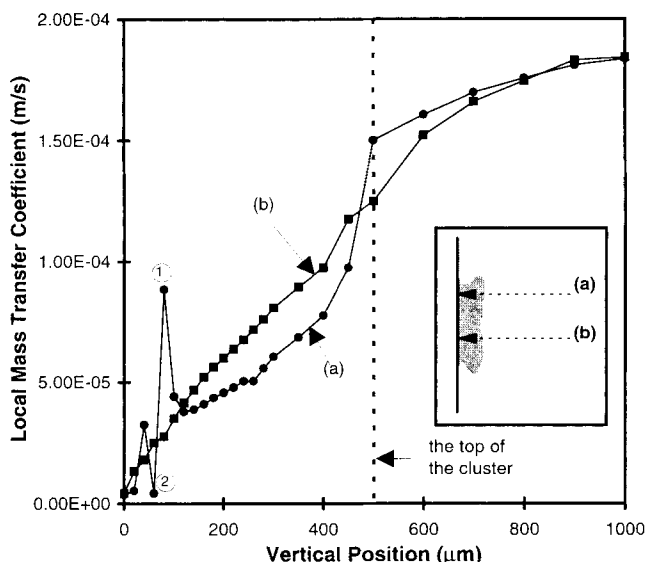


Figure 3. Vertical profiles of local mass transfer coefficient measured at two different locations through a cluster. The insert is the schematic diagram that shows locations of measurement. The shaded area represents biofilm clusters.

ducted in the reactor without biofilm (results not shown). No significant variations were observed.

The same measurements at various horizontal planes were conducted in the reactor with a 200- μm -thick biofilm cell cluster at a constant flow velocity, $V = 1.58 \text{ cm/s}$. Three horizontal distributions were taken in the bulk solution at different vertical positions to study the change of mass transfer rate in the bulk solution near the biofilm clusters, three other horizontal distributions were taken on or inside the biofilm matrix (Fig. 4a–f). For the six profiles, the corresponding distances from the biofilm substratum are: 1000, 500, 300, 200, 100, and 50 μm , respectively.

Mass Transfer Coefficient at Various Flow Velocities

The relationships between average mass transfer coefficient and the bulk flow velocity are presented in Figure 5. The average thickness of biofilm was estimated to be 120 μm . Measurements were conducted at three vertical positions: (1) at $z = 5000 \mu\text{m}$, in the bulk solution; (2) at $z = 100 \mu\text{m}$, just below the top of the biofilm; (3) at $z = 10 \mu\text{m}$, within the biofilm. Average mass transfer coefficients were calculated from horizontal distributions taken from a typical $250 \mu\text{m} \times 250 \mu\text{m}$ grid area. Curves (1) to (3) show the average mass transfer coefficients measured at these three vertical positions under various bulk flow velocities. The slopes of these curves indicated the sensitivity of mass

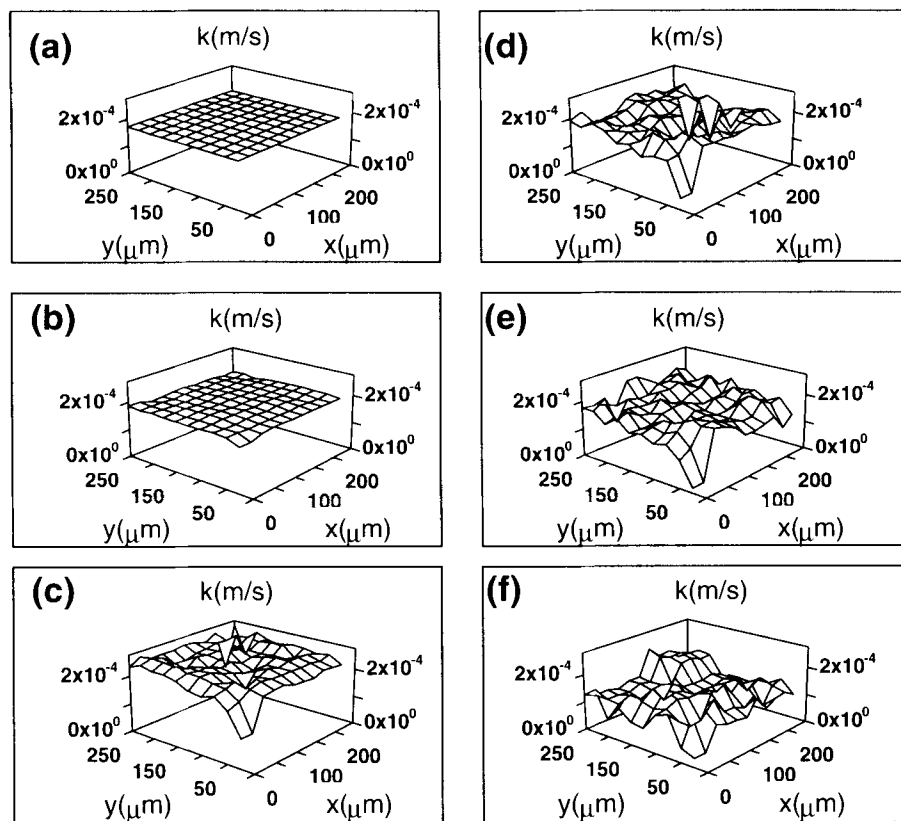


Figure 4. Horizontal distributions of local mass transfer coefficient. Bulk liquid flow velocity was 1.58 cm/s. (a) $z = 1000 \mu\text{m}$; (b) $z = 500 \mu\text{m}$; (c) $z = 300 \mu\text{m}$; (d) $z = 200 \mu\text{m}$; (e) $z = 100 \mu\text{m}$; and (f) $z = 50 \mu\text{m}$.

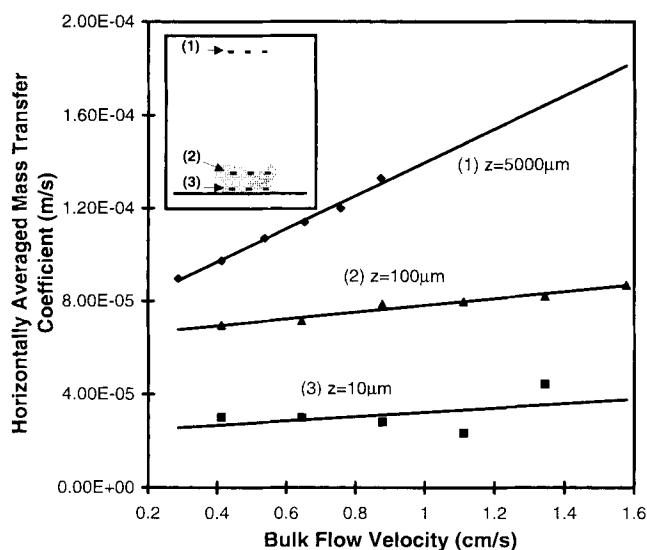


Figure 5. Influence of bulk flow velocity on horizontally averaged mass transfer coefficient. Insert: (1) measured in bulk liquid at $z = 5000 \mu\text{m}$; (2) measured in biofilm at $z = 100 \mu\text{m}$; and (3) measured in biofilm at $z = 10 \mu\text{m}$. The shaded area represents biofilm clusters. Dashed lines show where the horizontal distributions were taken from.

transfer coefficient to the change of flow velocity. The intercepts correspond to the mass transport rate in the stagnant water.

DISCUSSION

Applicability of LCT to Biofilm Systems

A major concern while applying LCT in biofilm systems was whether the biofilm structure would remain unchanged during the course of the measurement. The time required to complete a set of measurements ranged from 2 to 10 h. Observations by inverted microscope, supported by time-lapse photography, confirmed that biofilm structures remained intact throughout the measurements. In addition, the viable cell count demonstrated that biofilm microorganisms remained viable, despite the replacement of nutrients with $\text{Fe}(\text{CN})_6^{3-}$ solution during the experiments. Because the mass transfer rate of $\text{Fe}(\text{CN})_6^{3-}$ to the microelectrode was mainly influenced by the hydrodynamics and the biofilm structure, we concluded that LCT did not alter mass transfer process in biofilms, and could, therefore, be applied to biofilm systems.

Influence of Convective Flow Velocity on Overall Mass Transfer Coefficient

In these studies, the flow velocity used ranged from 0.2 to 1.6 cm/s and the corresponding range of Reynolds numbers ($Re = 4 \times V \times R_H/\nu$) was 50 to 500 where V is the liquid flow velocity, and ν is the liquid's kinematic viscosity. Therefore, results and conclusions reported here are only applicable to these flow conditions.

In the bulk flow, convective transport is the dominant

process of mass transport. From the results presented in curve (1) of Figure 5, the contribution of convective transport to the overall mass transfer coefficient was estimated. The mass transfer coefficient and the bulk flow velocity in the reactor were linearly related:

$$k(\text{m/s}) = 7.10 \times 10^{-3} \times V(\text{m/s}) + 6.83 \times 10^{-3} \quad (5)$$

From the slope of curve (1), $\Delta k/\Delta V = 7.10 \times 10^{-3}$, we can estimate the variation of convective flow velocity, ΔV , for the change of mass transfer coefficient, Δk , in channel flow.

The linear relationships between average mass transfer coefficient and bulk flow velocity hold inside the biofilm, as shown in curves (2) and (3) of Figure 5. The linear regressions for both curves reveal that the slopes and intercepts decrease as depth into the biofilm changes from 100 μm to 10 μm . The decreasing slope means that the influence of bulk flow velocity on the average mass transfer coefficient decreases toward the bottom of reactor, as expected. It also implies that essential nutrients for cell growth are more accessible in the top portion of biofilms than in the bottom, even though the liquid flows within the entire biofilm. The intercepts of the curves are the mass transfer coefficients at zero flow velocity. The change in the intercept may reflect a change in local molecular diffusion due to the density difference of biofilm matrix.

Analysis of Horizontal Distribution of Mass Transfer Coefficient

The results presented in Figure 4 show that the local mass transfer coefficient across a horizontal plane in a biofilm is not constant and its value is site specific. Calculated arithmetic average values and the standard deviations of the distributions in Figure 4 are given in Table II. Significant fluctuations of mass transfer coefficient occurred just above the top of solid biofilm matrix, $z = 200 \mu\text{m}$, and inside the biofilm, but not above a water channel.

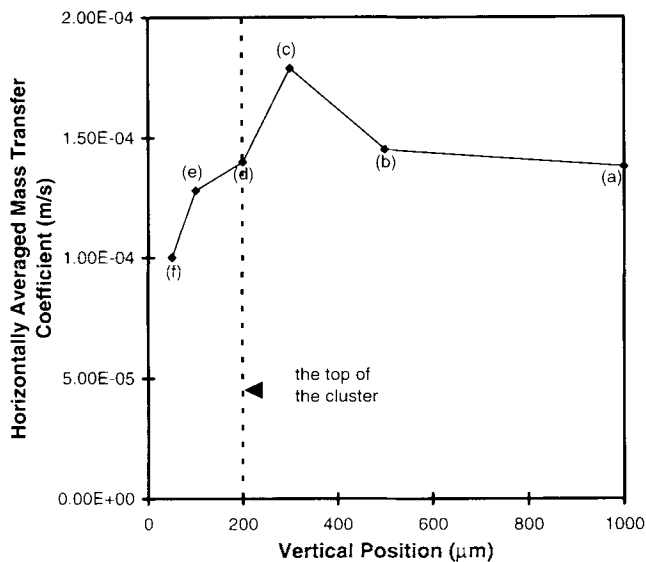
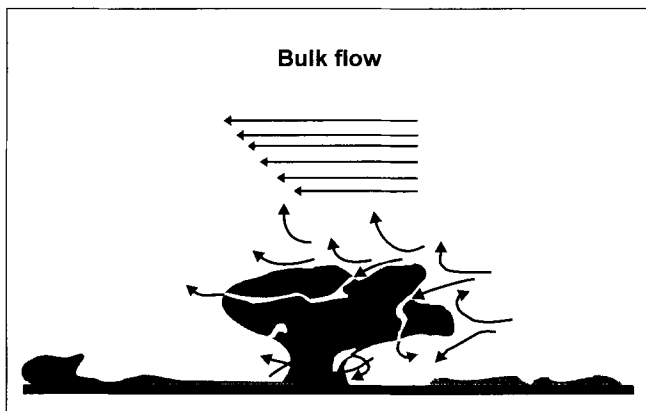
For a flow velocity of 1.58 cm/s, in the bulk flow at $z = 1000 \mu\text{m}$, the variation of mass transfer coefficient was 0.8%. The small variability is due to the stable bulk flow provided by the gravitational flow system. Just above the top of the biofilm solid matrix, from $z = 200$ to $500 \mu\text{m}$, both the average mass transfer coefficient and the standard variations of mass transfer coefficient increase considerably. The magnitude of the change can be seen more clearly by plotting k versus z (Fig. 6). This increase in mass transfer coefficient suggests the existence of increased mixing in this region. Turbulence could be the result of the interaction between structure of the biofilm matrix and hydrodynamics. Bioturbation^{16,20} (active movement of biofilm surface) could contribute to the increase in mass transfer rate.

Secondary Structural Heterogeneity of Biofilms

If mass transfer inside the biofilm matrix was dominated by molecular diffusion, the local mass transfer coefficient should be relatively constant. However, the horizontal dis-

Table II. Analysis of horizontal distributions in Figure 4.

Horizontal distributions	z, vertical position (μm)	k, average mass transfer coefficient (m/s)	δk , standard variation of k (m/s)	$\delta k/k$, percentage of variation (%)
(a)	1000	1.38e-4	9.72e-7	0.8
(b)	500	1.45e-4	3.21e-6	2.2
(c)	300	1.79e-4	2.10e-5	11.7
(d)	200	1.40e-4	2.66e-5	19.0
(e)	100	1.28e-4	2.56e-5	20.0
(f)	50	1.00e-4	2.93e-5	29.3

**Figure 6.** Vertical profile of horizontally averaged mass transfer coefficient calculated from Figure 4.**Figure 7.** The proposed structural model of biofilm cluster. Arrows indicate the direction of local liquid flow.

tributions presented in Figure 4e and f show that the local mass transfer coefficient values inside the biofilm were not constant and significant variations were observed. Results suggest a more complex mass transport mechanism than simple molecular diffusion inside biofilm clusters.

The vertical variation of mass transfer coefficient can be

seen from the vertical profiles presented in Figures 2 and 3. The sudden increases of mass transfer coefficient in curve (b) of Figure 2 and curve (a) of Figure 3 show that the local mass transfer coefficient can be unexpectedly high at some locations inside biofilm clusters. Similar measurements were carried out in uniform 3% calcium alginate layers and no such abrupt change in mass transfer coefficient was observed. Therefore, the possibility of transport of reacting species along the shaft of the microelectrode due to the movement of the microelectrode was considered insignificant. We suggest that the existence of channels facilitating convective flow within biofilm cell clusters is the major cause of the fluctuation in local mass transfer coefficient.

By using the relation established earlier for mass transfer coefficient and convective flow velocity [Eq. (5)], the change in convective flow velocity was estimated for the observed fluctuation of the local mass transfer coefficient. The difference of mass transfer coefficients, Δk , between point one and two in curve (a) of Figure 3 was 8×10^{-5} m/s. According to the slope of Eq. (5), the corresponding change in flow velocity, ΔV , is 1.13 cm/s. Such a change in convective flow velocity could be caused by a significant difference in physical structure between these two points in the biofilm cluster.

It is, therefore, hypothesized that the internal structure of the biofilm matrix is not uniform. A conceptual model of the biofilm matrix accounting for such a possibility is shown in Figure 6. The model assumes existence of flow channels with variable cross-sectional areas and irregular orientations inside biofilm clusters. Also, the irregular flow just above the top of the biofilm cluster is depicted to show the existence of turbulence in this region.

The flow channel inside biofilm clusters adds additional dimension to heterogeneity in the existing biofilm model. This model contributes to our understanding of biofilm heterogeneity and may also help explain some puzzling experimental results, such as that reported by Jansen and Kristensen,⁸ who measured a higher diffusivity in biofilm than in pure water. The results are not surprising when considering the convective mass transport inside the biofilm.

CONCLUSIONS

1. The local mass transfer coefficient in a biofilm is not constant. Its value varies horizontally and vertically.

2. The influence of hydrodynamics on the average mass transfer decreases toward the bottom of the biofilm.
3. The mass transfer coefficient increases just above the biofilm surface. The result suggests the existence of local flow instability in this region.
4. The vertical profile of the mass transfer coefficient measured across interstitial voids is similar to that measured in a sterile reactor.
5. Vertical profiles of mass transfer coefficients measured across biofilm cell cluster demonstrated unexplained variability. This variability was ascribed to the heterogeneous structure of biofilm cell clusters.

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