



Microelectrode measurement of local mass transfer coefficient in biofilms
by Shunong Yang

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in
Environmental Engineering
Montana State University
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Abstract:

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The limiting current technique was also used to measure local shear rate on microelectrode surface. A flow field model on the surface of circular-disk microelectrode was developed to describe the relationship between local mass transfer coefficient and local shear rate. Initial results show that local shear rate on the microelectrode surface increases exponentially with the increase of bulk flow velocity.

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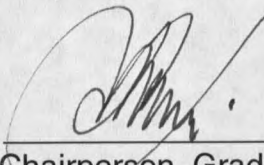
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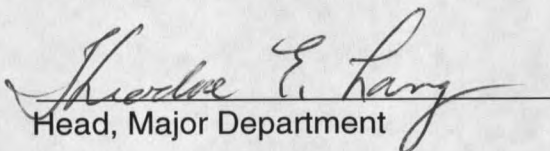
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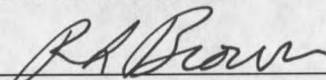
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TABLE OF CONTENTS

	Page
LIST OF TABLES	ix
LIST OF FIGURES	x
ABSTRACT	xii
INTRODUCTION	1
THEORY	7
Mass transfer coefficient calculation	7
Calculation of shear rate on electrode surface	9
Mathematical model	10
Solutions	11
MATERIALS AND METHODS	15
Biofilm growth	15
LCT experimental setup	16
Electrodes and electrolyte solution	16
Gravitational flow setup	18
LCT applicability tests	18
Measuring $\text{Fe}(\text{CN})_6^{3-}$ concentration in biofilms	19

Measuring limiting current at various $\text{Fe}(\text{CN})_6^{3-}$	
concentration.....	19
Monitoring biofilm structure	19
Local mass transfer coefficient measurements.....	20
Measuring vertical profiles of local mass transfer	
coefficient.....	20
Measuring horizontal distributions of local mass transfer	
coefficient	22
Combination of CSLM velocimetry and LCT	
measurements.....	24
RESULTS	26
$\text{Fe}(\text{CN})_6^{3-}$ concentration distribution of in biofilms	26
Independence of mass transfer coefficient on $\text{Fe}(\text{CN})_6^{3-}$	
concentration	26
Biofilm structure and viability	26
Vertical profiles of local mass transfer coefficient	27
3%calcium alginate layer	27
Biofilms	28
Horizontal distribution of local mass transfer coefficient	29
Mass transfer coefficient at various flow velocities	29

Vertical profiles of local velocity and mass transfer coefficient in biofilm void	30
DISCUSSION	43
Experimental conditions influencing mass transfer coefficient.....	43
Microelectrode cleaning	43
Reacting electrolyte selection	44
Applicability of LCT to biofilm system	45
Estimation of measurement errors on mass transfer coefficient	46
Possible error in measuring sensing area of microelectrode	46
Possible error in measuring bulk $\text{Fe}(\text{CN})_6^{3-}$ concentration	47
Possible error in measuring limiting current	47
Other possible error	47
Influence of convective flow velocity on mass transfer coefficient	48
Analysis of horizontal distribution of local mass transfer coefficient	49
Secondary structural heterogeneity of biofilms	51

Local shear rate on microelectrode surface	53
Correlation of local flow velocity and local mass transfer coefficient	54
SUMMARY	60
RECOMMENDATION FOR FUTURE RESEARCH	62
REFERENCES	63
NOMENCLATURE	69
APPENDIX	71

LIST OF TABLES

Table		Page
1.	Viabie cell counts of the biofilm under LCT measurement condition	27
2.	Analysis of horizontal distribution in Fig. 10	50

LIST OF FIGURES

Figure		Page
1.	The existing steady-state biofilm model	2
2.	Flow field model on the surface of circular-disk microelectrode	10
3.	Relationship between Peclet number and Sherwood number for circular-disk microelectrode	14
4.	The schematic diagram of LCT experimental setup	25
5.	Mass transfer coefficient vs. $\text{Fe}(\text{CN})_6^{3-}$ concentration	32
6.	Vertical profile of mass transfer coefficient through a alginate layer	33
7.	Vertical profile of mass transfer coefficient through a alginate layer	34
8.	Vertical profiles of local mass transfer coefficient taken in a biofilm void (a) and through a biofilm cluster (b)	35
9.	Vertical profiles of local mass transfer coefficient measured at two different locations in the same biofilm cluster	36
10.	Horizontal distributions of local mass transfer coefficient at $V=1.59\text{cm/sec}$	37&38

11.	Horizontal distributions of local mass transfer coefficient at $z=50\text{ }\mu\text{m}$ under various flow velocity.	39
12.	Influence of bulk flow velocity on horizontally averaged mass transfer coefficient at $z=50\text{ }\mu\text{m}$	40
13.	Influence of bulk flow velocity on horizontally averaged mass transfer coefficient at three vertical depths	41
14.	Vertical profiles of local flow velocity and local mass transfer coefficient in a biofilm void	42
15.	Vertical profile of horizontally averaged mass transfer coefficient through a biofilm cluster	55
16.	Vertical profile of horizontally averaged mass transfer coefficient for a $200\text{ }\mu\text{m}$ high biofilm cluster	56
17.	Proposed structural model of biofilm clusters	57
18.	Relationship between local shear rate on circular-disk microelectrode and bulk flow velocity	58
19.	Correlation between local mass transfer coefficient and local flow velocity in a biofilm void	59

ABSTRACT

Recent studies revealed that biofilm structures are heterogeneous. The immediate consequence of this structural heterogeneity is the non-uniformity of local mass transfer processes in biofilms. So far, no theoretical or experimental approach has been provided to describe it.

This thesis presents an experimental technique to measure local mass transfer coefficients in biofilms. Local mass transfer rates in an aerobic biofilm was measured using a limiting current technique in an electrochemical microsink formed by a mobile microelectrode. Viable cell count and time-lapse photography results show that the technique is applicable to biofilm system. Local mass transfer coefficients calculated from the measured limiting current varied both horizontally and vertically in the biofilm. Mapping of the biofilm with the electrode verified the existence of an irregular biofilm structure comprised of microbial clusters surrounded by torturous water channels. Unexpected increases of local mass transfer coefficient within 300 μm above biofilm surface suggested the existence of local turbulence 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 biofilm cell cluster, suggesting the existence of internal channels. A new conceptual model of biofilm cell cluster structure is proposed to account for such biofilm microstructure irregularities.

The limiting current technique was also used to measure local shear rate on microelectrode surface. A flow field model on the surface of circular-disk microelectrode was developed to describe the relationship between local mass transfer coefficient and local shear rate. Initial results show that local shear rate on the microelectrode surface increases exponentially with the increase of bulk flow velocity.

INTRODUCTION

Steady-state biofilm models^{9, 32, 33} commonly assume that internal mass transfer (inside biofilms) is diffusion controlled and that external mass transfer (outside biofilms) is dominated by convection. Consequently, it is believed that the diffusivity is the only parameter needed to describe mass transfer rate inside biofilms. Mass transfer and reaction are thus incorporated into a mass balance equation as follows:

$$D \frac{d^2 C}{dz^2} = \frac{\mu_m C C_x}{K_c + C} \quad (1)$$

The mass transfer rate from the bulk fluid to the biofilm surface is commonly approximated by assuming that the mass boundary layer above the biofilm surface is uniform and stagnant. Thus, the flux across the external mass transfer layer is treated as a boundary condition and can be expressed as

$$J = D \times \frac{\Delta C}{\delta} = k \times \Delta C \quad (2)$$

A descriptive diagram of the existing biofilm steady state model is shown in Fig. 1.

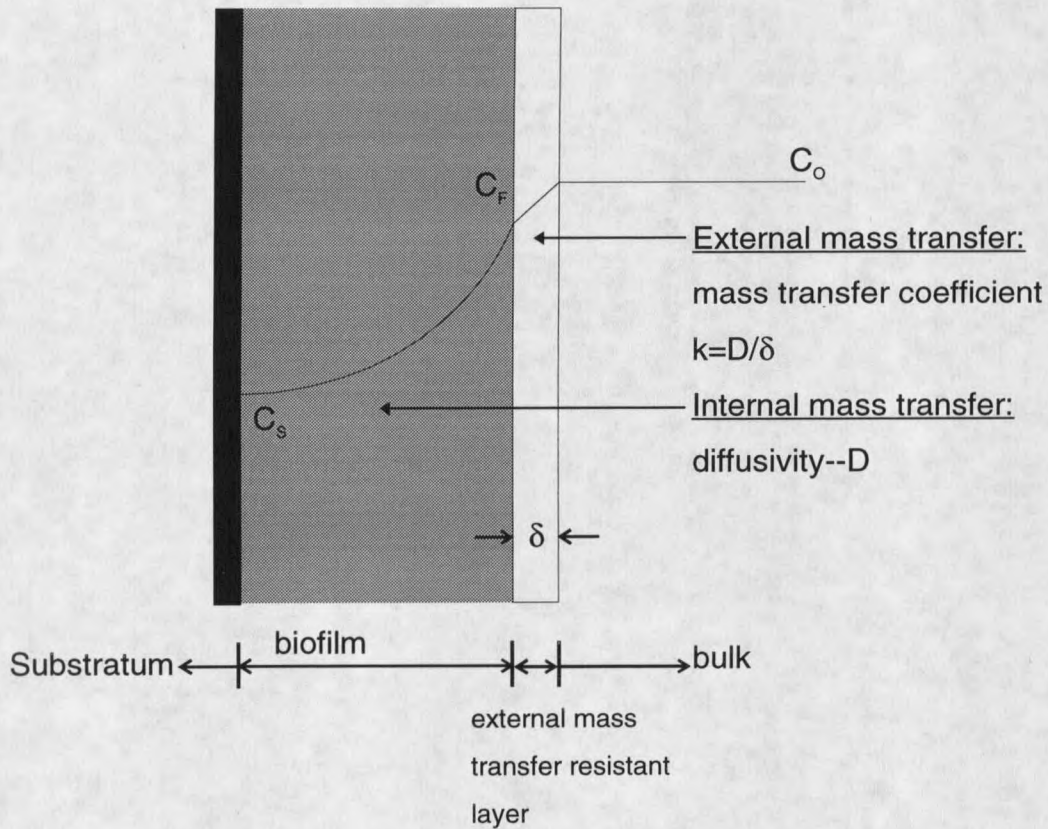


Fig. 1 The existing steady-state biofilm model

This simple mass transport model in biofilms has been challenged by the observations of Light Microscopy (LM)^{3,11,19}, Atomic Force Microscopy (AFM)², Scanning Electron Microscopy (SEM)^{7,26}, and Confocal Scanning Laser Microscopy (CSLM)^{5,6,39}. Recent studies by CSLM reveal that the structures of aerobic biofilms are not uniform but consist of microbial cell clusters separated by interstitial voids. According to this concept, mass transport in interstitial voids is mainly facilitated by convective flow while mass transfer inside 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 can no longer be considered the same at different locations in a biofilm due to variable porous structure and subsequent reactivities that vary in space. Rather, mass transfer rates depend on complex interactions among local biofilm activity, structure, and hydrodynamics. The situation is even more complicated because that biofilms are viscoelastic and microscale hydrodynamics near such surfaces has not been characterized analytically. Since 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 relationship between biofilm structure and hydrodynamics. Lewandowski *et al*²¹ described liquid flow in a biofilm reactor using Nuclear Magnetic Resonance Imaging (NMRI) with limited spatial resolution. De Beer *et al*⁶ and Stoodley *et al*³⁹ measured liquid flow velocity in biofilm voids by using Particle Image Velocimetry (PIV) which traces the horizontal movement of fluorescent beads in the voids of biofilms using CSLM. While these papers quantify factors influencing mass transport rate, none of them provides the means to actually measure it.

This thesis describes measurements of local mass transfer coefficients in biofilms using a modified Limiting Current Technique (LCT). LCT uses a particular electrochemical reaction that proceeds at the highest 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.

Historically, Lin *et al*²² made a systematic study of mass transfer rates of ions and other reacting species in electrochemical processes and measured the mass transfer coefficients in laminar and turbulent flows using LCT. Ranz²⁹ discussed problems associated with applying LCT to liquid flow velocity measurement. Mitchell and Hanratty^{13,24} developed LCT into a wall shear-stress meter and used it in the study of turbulence at a wall. D.A.Dawson and O.Trass⁴ used LCT in the study of mass transfer rates at rough surfaces. The basic principle and applications of the conventional LCT were reviewed in several publications^{25, 35, 41}.

This well-known technique was also used to study local mass transfer rates^{8, 16, 17, 18,34} to flat surfaces. The electrodes used were flush mounted to the reactor's wall. Sizes of electrodes range 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; (2) the size of the electrode was reduced to a few micrometers to prevent damaging the biofilm structure and disturbing the hydrodynamics.

The tip of the microelectrode formed a microsink for the electroactive species purposely added to the reactor. Measured limiting current was directly proportional to the local mass transfer rate at the tip of the microelectrode. The magnitude of the limiting current was dependent on a combination of all factors influencing the local mass transfer rate, e.g., the local hydrodynamics and the local structure of the biofilms. Consequently, the local mass transfer coefficient calculated from the measured limiting current to the microelectrode is related to the local effective diffusivity that is a function of local hydrodynamics and local biofilm structure.

Hydrodynamic flow in biofilm reactors does not just influence mass transfer rate. Another important aspect of its influence is on biofilm shearing, i.e., the loss of biomass from biofilm reactor as a result of shear forces. The mechanism of shearing loss is still unknown due to its complexity. Because of differences in time scale and types of biofilms and reactors investigated, some completely contradictory results have been drawn from various studies. Gantzer *et al*¹⁰ concluded that higher biomass levels in streambed biofilms were found at the faster acclimation velocities. Lau and Liu²⁰ discovered that biofilm biomass accumulation was substantially reduced as flow shear stress increased and that the maximum accumulation occurred under very low flow conditions. To understand the mechanism of biofilm shearing loss, it is very important to measure local shear stress on biofilm surface under the defined hydrodynamic

condition and correlate local shear stress data with biofilm accumulation or biofilm detachment. LCT is a potential tool for acquiring such information. Besides measuring local mass transfer coefficient, application of LCT has been extended by Hanratty *et al*¹² to measure wall shear stress on the surface of sensing electrodes. With a hydrodynamic model for the flow field on the surface of an electrode, the relationship between wall shear stress and mass transfer coefficient can be established. A flow field model and its solutions for the circular-disk microelectrode will be discussed in the next chapter.

THEORY

Mass transfer coefficient calculation

During LCT measurement, the ions reacting on the sensing electrode are transferred from the bulk of the solution to the surface of the electrode principally by (a) migration due to the potential field, (b) diffusion due to the concentration gradient, and (c) convective flow. $\text{Fe}(\text{CN})_6^{3-}$ is one of the commonly used reacting species in LCT. The electrochemical reduction of $\text{Fe}(\text{CN})_6^{3-}$ occurs on the surface of the microelectrode as



By adding an excess amount of non-reactive supporting electrolyte, e.g., KCl, to the electrolyte solution, there should be little potential gradient formed near electrode surface. Thus, the electromigration process becomes negligible and almost all of the electrolyte current arises from the reaction of ions that reach the electrode surface by diffusion and/or convection. The flux of the reacting species onto the electrode surface is related to mass transfer coefficient, k ,

$$J = k(C_o - C_s) \quad (4)$$

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 ferricyanide ion, J , to the surface of the microelectrode with sensing area A is related to the limiting current, I

$$J = \frac{I}{nAF} \quad (5)$$

The relation between mass transfer coefficient and limiting current can be found by combining Eqn. (4) and (5) for $C_s=0$

$$k = \frac{I}{nAFC_o} \quad (6)$$

With the elimination of migration effect, the information obtained from LCT can be applied to non-electrochemical process. Furthermore, the analogy between heat and mass transfer becomes valid as discussed by Agar¹. Thus mass transfer results obtained by LCT may be correlated with the knowledge of heat transfer.

Calculation of shear stress (τ) at the electrode surface

As pointed out in the previous chapter, LCT has a potential application in investigating local shear stress in biofilm reactors. To obtain local shear stress, a flow field model on the microelectrode surface has to be established.

Due to the fabrication capability and experimental convenience, the tip surface of the microelectrode used in this study was a circular disk with its diameter on the order of a few micrometers. The flow field on the surface of circular-disk microelectrode is illustrated in Fig. 2.

The basic assumptions implied in this figure are:

- (a) the scalar boundary layer is within the region where $V=Sz$;
- (b) the flow is homogeneous over the electrode surface;
- (c) the velocity components other than in the Z-direction are negligible.

Generally, for large Sc and small A , the concentration boundary layer over the microelectrode will be so thin that it lies within a region in which the velocity field is a simple shear flow with a constant shear rate.

Theoretical issues of above assumptions have been discussed in detail by Phillips²⁷, Hanratty and Campbell¹³ and Stone³⁸.

Mathematical model

The steady-state mass balance equation on the electrochemical reacting species for the described flow field can be written as

$$D\nabla^2 C = S_z \frac{\partial C}{\partial X} \quad (7)$$

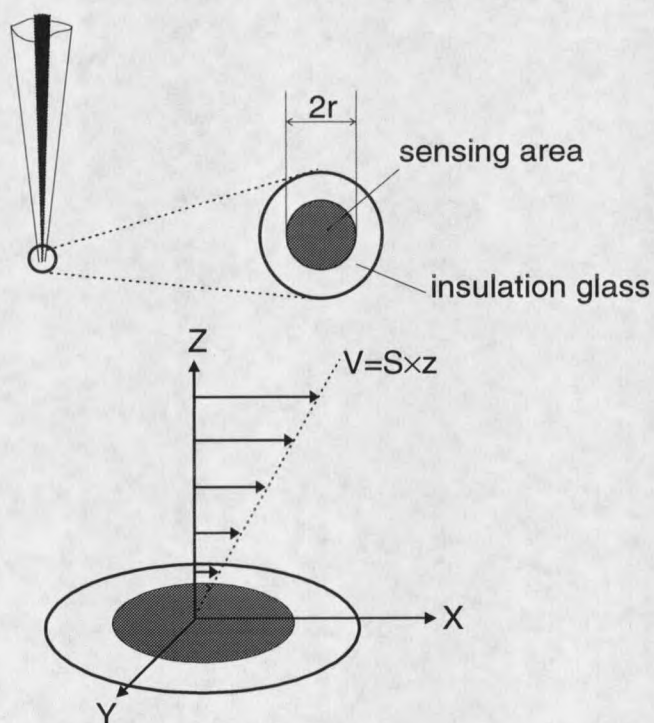


Fig. 2 Flow field model on the surface of circular-disk microelectrode

The boundary conditions are

$$C=0 \text{ for } z=0, \phi = r$$

$$\frac{\partial C}{\partial Z} = 0 \quad \text{for } z=0, \phi > r$$

$$C = C_o \quad \text{as } (x^2 + y^2 + z^2)^{1/2} \rightarrow \infty$$

The equation can be written in terms of dimensionless form

$$\nabla^2 \theta = Pe \eta \frac{\partial \theta}{\partial \xi} \quad (8)$$

By solving the mass balance Eqn. (8), The relationship between dimensionless mass transfer coefficient, Sh, and dimensionless shear rate, Pe, can be established.

Solutions

Analytical solution by Reiss and Hanratty³⁰: By neglecting diffusions in X- and Y-directions, Eqn. (8) is simplified

$$\frac{\partial^2 \theta}{\partial \eta^2} = Pe \eta \frac{\partial \theta}{\partial \xi} \quad (9)$$

Eqn. (9) was solved analytically and a relation between Sherwood number and Peclet number was obtained

$$Sh = 0.68658 Pe^{1/3} + O(Pe^{-1/6}) \quad (10)$$

or the shear rate can be calculated explicitly from $S=1.45649 \times k \times r^{1/3} \times D^{-2/3}$. The numerical solution obtained by Stone³⁸ for Eqn. (8) shows that the error in using Eqn. (10), because of neglecting diffusions in X- and Y-directions, is less than 5% for $Pe > 400$. The high Pe situation can be encountered when sensor diameter is big, and/or velocity gradient is large, and/or diffusivity of reacting species is small.

Analytical solution by Phillips²⁷: The limitation of using Eqn. (10) lies in its one dimension diffusion assumption. Generally, the diffusion problem is fully three dimensional or the edge effect cannot be neglected. By using the method of matched asymptotic expansions, Phillips solves Eqn. (8) analytically. The final solution is written as

$$Sh = \frac{4 - 0.11268 Pe^{3/2}}{\pi(1 - 0.20281 Pe^{1/2})} \quad (11)$$

Results calculated from Eqn. (11) were compared with Stone's numerical solutions. Both results agree within 2% from $Pe=0.001$ up to $Pe=2$. The smallness of Pe means that on the scale of microelectrode surface, convection, represented by the right-hand side of Eqn. (8), is weak. It results in a small perturbation of a purely diffusive solution. This solution is applicable when the microelectrode is used in low velocity flow.

Numerical solution by Stone³⁸: Numerically, Stone evaluated the mass transfer rate over a wide range of Peclet numbers ($0.001 < Pe < 400.0$). Without making additional assumption to the problem represented in Eqn. (8), Stone used one of the boundary integral methods to calculate overall mass transfer rate at different Pe values. By performing the regression on his numerical results, Stone proposed a correction term for Eqn. (10) at high Peclet number ($Pe > 10$). The new equation is written as

$$Sh = 0.68658 Pe^{1/3} + 1.13000 Pe^{-1/6} \quad (12)$$

The results calculated from this equation have good agreement with the numerical solution in the high Peclet number range ($10 < Pe < 400$).

To calculate Peclet number from Sherwood number, or shear rate from mass transfer coefficient, for a wide range of Peclet numbers (from 0.001 to 400), it will be very convenient to have a single analytical formula that applies to the entire Pe range. Eqn. (11) fits the numerical solution very well up to $Pe=2$ and results given by Eqn. (12) lie within 3% of the numerical solution for Peclet numbers as low as 10. By fitting the data provided by Eqn. (11) from $Pe=0.001$ to 2 and Eqn. (12) from 10 up to 400, we generated Eqn. (13) that is valid Pe from 0.001 to 400. A curve fitting software, TableCurve 2D of Jandel Scientific, was used.

$$\sqrt{Pe} = -20.5 + 40.3 \times Sh - 31.7 \times Sh^2 + 12.9 \times Sh^3 - 2.47 \times Sh^4 + 0.183 \times Sh^5 \quad (13)$$

Solutions of Eqns. (11), (12), and (13) are presented in Fig. 3.

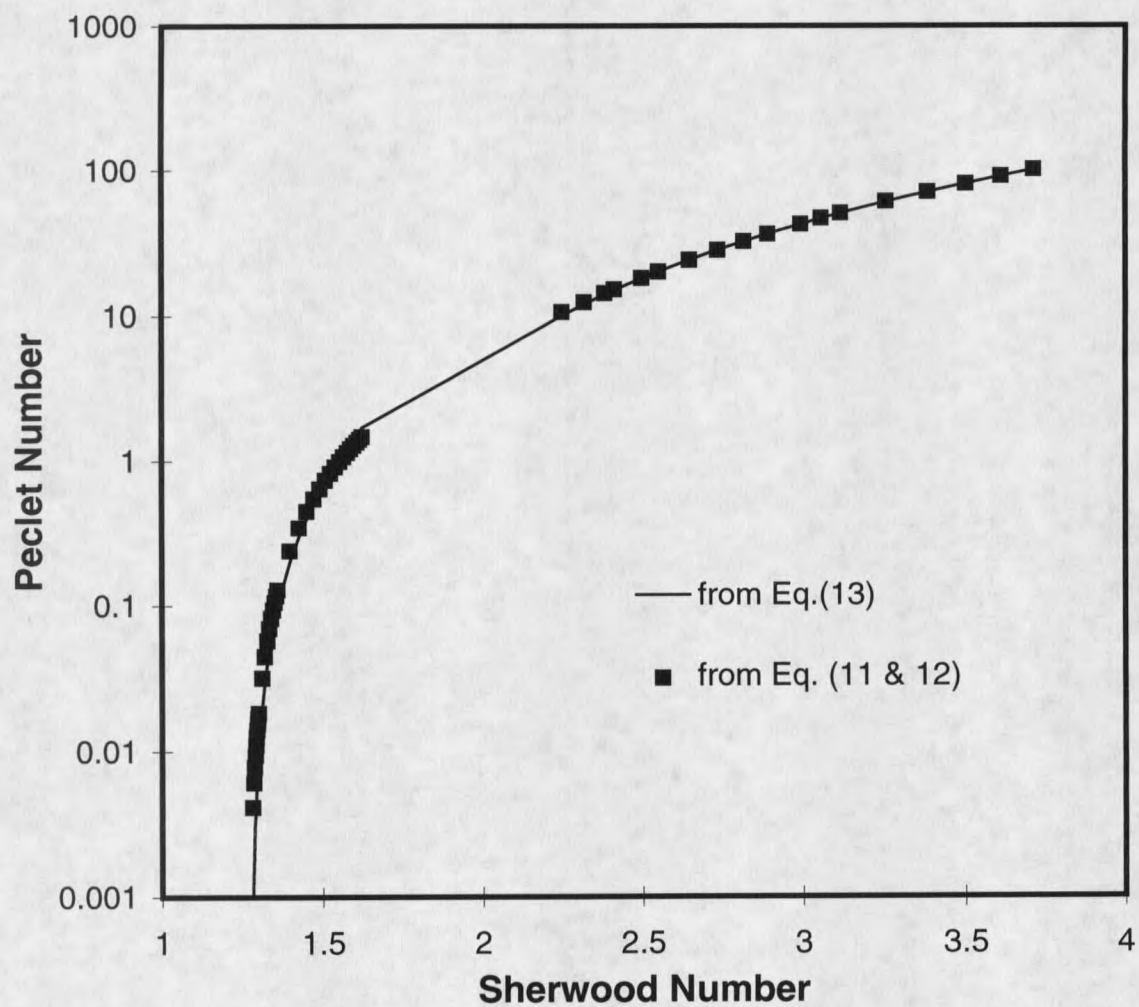


Fig. 3 Relation between Peclet number and Sherwood number for a circular-disk microelectrode

MATERIALS AND METHODS

Biofilm growth

The 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. Nutrient solution consisting 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) 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 pre-aerating 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 the aerobic organisms: *Pseudomonas aeruginosa*, *Pseudomonas fluorescens* and *Klebsiella pneumoniae*. Use of this defined mixed population to form biofilms was described by Stoodley *et al*³⁹. After 12 h of batch operation, the reactor was switched to a continuous flow mode. Nutrient solution 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. 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 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.

LCT experimental setup

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 2M 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 Micro Electrode Puller (Stoelting Co., Wooddale, IL.) with the tip of the wire 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). Surface of

the microelectrode was cleaned in a sonication bath first with deionized water, then with acetone. Tip diameter was measured under the microscope and the surface area of the electrode calculated. Electrical connection with 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 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 with limiting current in the voltage range between -0.6 to -0.9 V were considered useable.

The reaction/diffusion solution was an electrolyte solution consisting of 0.5 M of KCl and 25 mM of $K_3Fe(CN)_6$. 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 viscometer (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 titration⁴⁰ before each measurement.

Gravitational flow setup

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

LCT applicability tests

In order to apply LCT to measure local mass transfer coefficients in biofilms, a few critical conditions need to be met. They are: (1) the concentration of $\text{Fe}(\text{CN})_6^{3-}$ is uniformly distributed in biofilm reactor or its local concentration can be measured, (2) the calculated mass transfer coefficient is independent of the concentration of $\text{Fe}(\text{CN})_6^{3-}$ used in the measurement, and (3) biofilm structure remains intact during the measurement.

Measuring $\text{Fe}(\text{CN})_6^{3-}$ concentration in biofilms

The tip of a microelectrode described in the Electrodes and electrolyte solution section was coated with a thin layer of cellulose acetate membrane so that it was not sensitive to mass transfer rate but only to the concentration of $\text{Fe}(\text{CN})_6^{3-}$. The electrolyte solution was allowed to recycle through the biofilm reactor for 30 min. Then vertical profiles of $\text{Fe}(\text{CN})_6^{3-}$ concentration in biofilms were measured by using this $\text{Fe}(\text{CN})_6^{3-}$ sensing microelectrode.

Measuring limiting current at various $\text{Fe}(\text{CN})_6^{3-}$ concentrations

For the electrolyte solutions used in the experiment, the concentration of KCl was kept constant and the concentration of $\text{Fe}(\text{CN})_6^{3-}$ was varied from 2.5mM to 50mM. Under the same flow velocity $V=2.2$ cm/sec, a microelectrode was positioned in the bulk flow and limiting current was measured for different concentration of $\text{Fe}(\text{CN})_6^{3-}$. A relation between the measured mass transfer coefficients and $\text{Fe}(\text{CN})_6^{3-}$ concentrations was obtained.

Monitoring biofilm structure

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, micro-photographs of biofilm were taken hourly to check for structural integrity of the

biofilms and samples of biofilm were taken for R2A agar plate counts to determine the viability of biofilm at time 0, 2, 4, and 22 h.

Local mass transfer coefficient measurements

Measuring vertical profiles of local 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, Stratford, CT). The microelectrode was introduced from the top of the reactor. To ensure that the viscous flow profile at the location of the measurement was fully developed, the distance from chosen measurement location to the entrance of reactor was always larger than the hydrodynamic entrance length as estimated according to Schlichting's equation¹⁴, $L=0.04 \times R_H \times Re$, where R_H is the hydraulic radius in centimeters and Re is the Reynolds number.

The microelectrode was introduced into the biofilm perpendicularly to the bottom of the reactor. Location of the tip in the biofilm was monitored through an inverted microscope. To measure the vertical profiles of 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 distance between two adjacent positions, for microelectrodes with a tip diameter of 10 μm , was set greater than 20 μm .

To establish the vertical Z-coordinate, the position of the reactor's bottom plate (the substratum for the biofilm) was arbitrarily set as $z=0$. The vertical position of the microelectrode, z , was then measured as the distance from tip of 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 the computer's 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 and compared with a preset standard deviation defining the desired precision of the measurement. If the measured standard deviation was smaller than the preset, the measurement was accepted, the data stored, result displayed on the screen, and the microelectrode moved to the next vertical position. If the calculated standard deviation was higher than the preset, the measurement was repeated. A compromise between the precision and practicality had to be accepted to

complete the measurement in a reasonable time. A standard deviation of 5% was set for all measurements.

To study mass transfer rate over a homogeneous viscoelastic surface and to test the performance of the technique, a layer of 3% calcium alginate was fixed on the bottom of the reactor. The thickness of the layers varied from 300 to 700 μm . Vertical profiles of limiting current through alginate layers were measured.

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

Measuring horizontal distributions of local mass transfer coefficient

Fig. 4 is the schematic diagram of the experimental setup. 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, Laguna Hills, CA) and positioned above the reactor. The micropositioners were computer controlled through a controller (Model CTC-283-3, Micro Kinetics, Laguna Hills, CA) 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 certain area. The usual grid setting was a 10×10 matrix, with step size of $25 \mu\text{m}$ in both horizontal directions. A similar data acquisition protocol used in the vertical profile measurement was applied. The microelectrode was first manipulated manually to the first grid point at a predetermined position in the biofilm. Once 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 predestined 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 measurements were 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.

Combination of CSLM velocimetry and LCT measurements

An experiment was set up to measure local velocity profile and local mass transfer coefficient profile at the same location in biofilm voids. The similar setup described in Monitoring biofilm structure was used. Instead of using a conventional inverted microscope, a CSLM was used. Local velocity profile in biofilm voids was measured by PIV^{6,39}. The vertical profile of local mass transfer coefficients was measured at the exact same location using LCT.

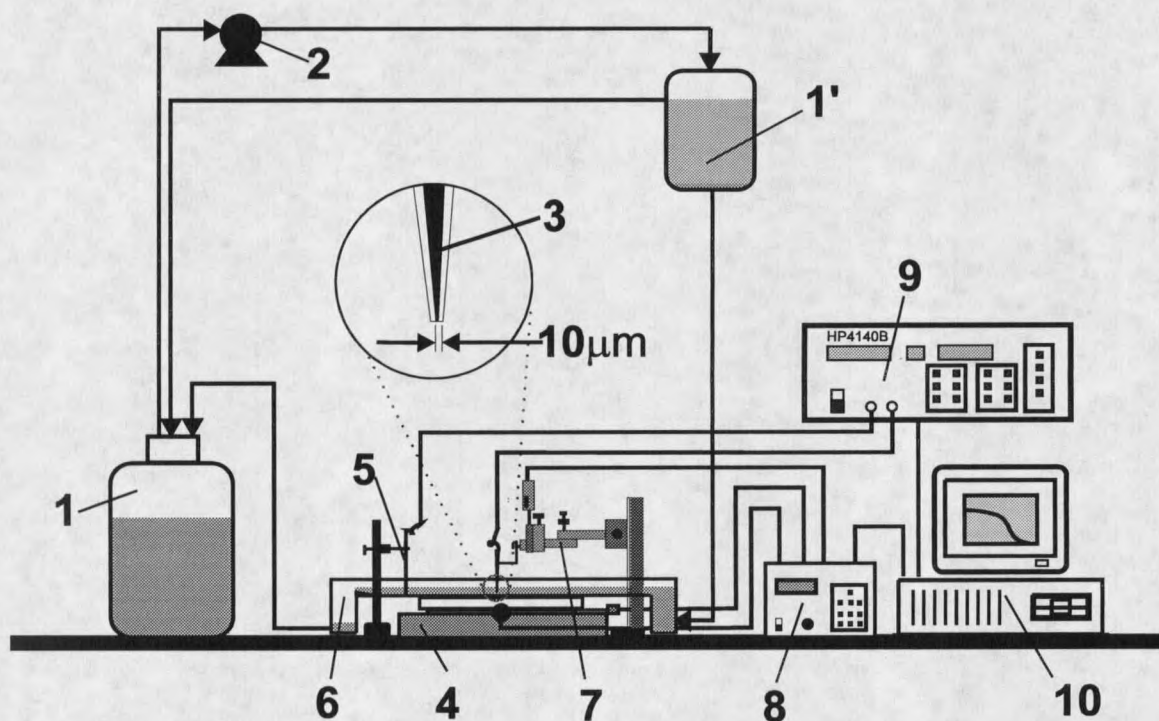


Fig. 4 The schematic diagram of LCT experimental setup. 1,1' -- electrolyte reservoir, 2 -- pump, 3 -- microelectrode, 4 -- X, Y microposition stage, 5 -- reference/counter electrode, 6 -- open channel biofilm reactor, 7 -- linear micropositioner, 8 -- micropositioning controller, 9 -- potentiostat, 10 -- computer.

RESULTS

$\text{Fe}(\text{CN})_6^{3-}$ concentration distribution in biofilms

By using the concentration sensitive microelectrode, $\text{Fe}(\text{CN})_6^{3-}$ concentrations were measured at various locations in the biofilm reactor and were found constant throughout the reactor.

Independence of mass transfer coefficient on $\text{Fe}(\text{CN})_6^{3-}$ concentration

Limiting current was measured at various ferricyanide concentrations. Results are plotted in Fig. 5. By changing the concentration of $\text{Fe}(\text{CN})_6^{3-}$ from 2.5 mM to 50 mM, the measured limiting current increased linearly with the concentration as shown. For each concentration, a mass transfer coefficient was calculated from the corresponding limiting current according to Eqn. (6).

Biofilm structure and viability

Microscopic observation supported by time-lapse photography revealed that the biofilm overall structure remained intact for more than 20 hours 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, results of plate counts showed that the number of viable cells in the biofilm remained relatively constant in the first four hours. Table 1 presents the results of the viable cell count for biofilm samples taken as a function of time since the onset of electrolyte recycling.

Table 1. Viable cell counts of the biofilm under LCT measurement condition

Time of sampling (hr)	0	2	4	22
Viable cell count (CFU/cm ²)	4.8×10^7	3.6×10^8	1.2×10^8	1.2×10^4

Vertical profiles of local mass transfer coefficient

3% calcium alginate layer

Fig. 6 and 7 are vertical profiles taken through uniform alginate layers. The bulk flow velocity was 1.15 cm/sec. The thickness of the alginate layer was 460 μm for Fig. 6 and 650 μm for Fig. 7. A significant decrease of local mass transfer coefficient was observed in both profiles when the microelectrode penetrated the surface of the alginate layer.

Biofilms

Fig. 8 illustrates two typical vertical profiles of the local mass transfer coefficient. Curve (a) was measured in a water-filled 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 as the local fluid flow velocity decreased. Curve (b) was measured through a 170 μm thick biofilm cluster. For vertical positions above $z=700\text{ }\mu\text{m}$, the local mass transfer coefficient held constant at the pure fluid value. Between $z=400$ and $700\text{ }\mu\text{m}$, a decrease of the local mass transfer coefficient was observed. Just above the top of the cluster, between $z=200$ and $400\text{ }\mu\text{m}$, the local mass transfer coefficient increased slightly. Inside the biofilm cluster, the local mass transfer coefficient fluctuated significantly.

In a separate experiment, two profiles presented in Fig. 9 were taken at two horizontal locations, 200 μm apart, in a 450 μm thick section of biofilm. Profile (a) demonstrates a randomly fluctuating mass transfer coefficient similar to that observed in Fig. 8. Profile (b) shows a rather smooth decrease in the local mass transfer coefficient.

Horizontal distribution of local mass transfer coefficient

Measurements of the local mass transfer coefficient at various vertical positions above a horizontal plane were conducted in the reactor without biofilm (results not shown). No significant variation was observed within each individual distribution.

Similar measurements at various horizontal planes were conducted in the reactor with a 200 μm thick biofilm cluster at a constant flow velocity, $V=1.58$ cm/sec. Three horizontal distributions were taken in the bulk solution at different vertical positions in the bulk solution to study the change of mass transfer rate above biofilm clusters. Three other horizontal distributions were taken on or inside the biofilm matrix. These six distributions are presented as Figures 10a to 10f. Their corresponding vertical positions above the biofilm substratum are: 1000, 500, 300, 200, 100, 50 μm , respectively.

Mass transfer coefficient at various flow velocities

Fig. 11 presents the change in horizontal distributions of local mass transfer coefficients in biofilms corresponding to the change in flow velocity. For a 200 μm thick biofilm cell cluster, horizontal distributions of local mass transfer coefficients were taken at $z=50$ μm . The flow velocity was varied from 0.41

cm/sec to 1.4 cm/sec. For each distribution, the horizontally averaged value of the mass transfer coefficient was calculated and plotted against the flow velocity in Fig. 12.

In a separate experiment, horizontal distributions of local mass transfer coefficient were measured at various vertical positions and various flow velocities. The average mass transfer coefficient for each distribution was calculated. The relationships between average mass transfer coefficient and the bulk flow velocity are presented in Fig. 13. The average thickness of biofilm under investigation was estimated at 120 μm . Measurements were conducted at three vertical positions: (1) $z=5000\text{ }\mu\text{m}$ in the bulk solution; (2) $z=100\text{ }\mu\text{m}$ just below the top of the biofilm; (3) within the biofilm at $z=10\text{ }\mu\text{m}$. Average mass transfer coefficients were calculated from horizontal distributions taken from a typical $250\text{ }\mu\text{m}\times 250\text{ }\mu\text{m}$ 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 transfer coefficient to the change in flow velocity. The intercepts provided mass transport information in no convective flow condition.

Vertical profiles of local velocity and mass transfer coefficient in void

At the same location in a biofilm void, the vertical profile of local flow velocity and the vertical profile of local mass transfer coefficient were measured

and presented in Fig. 14. Both local velocity and local mass transfer coefficient decreased toward the base of the biofilms, but the rate of decrease of local flow velocity was much more significant than that of local mass transfer coefficient.

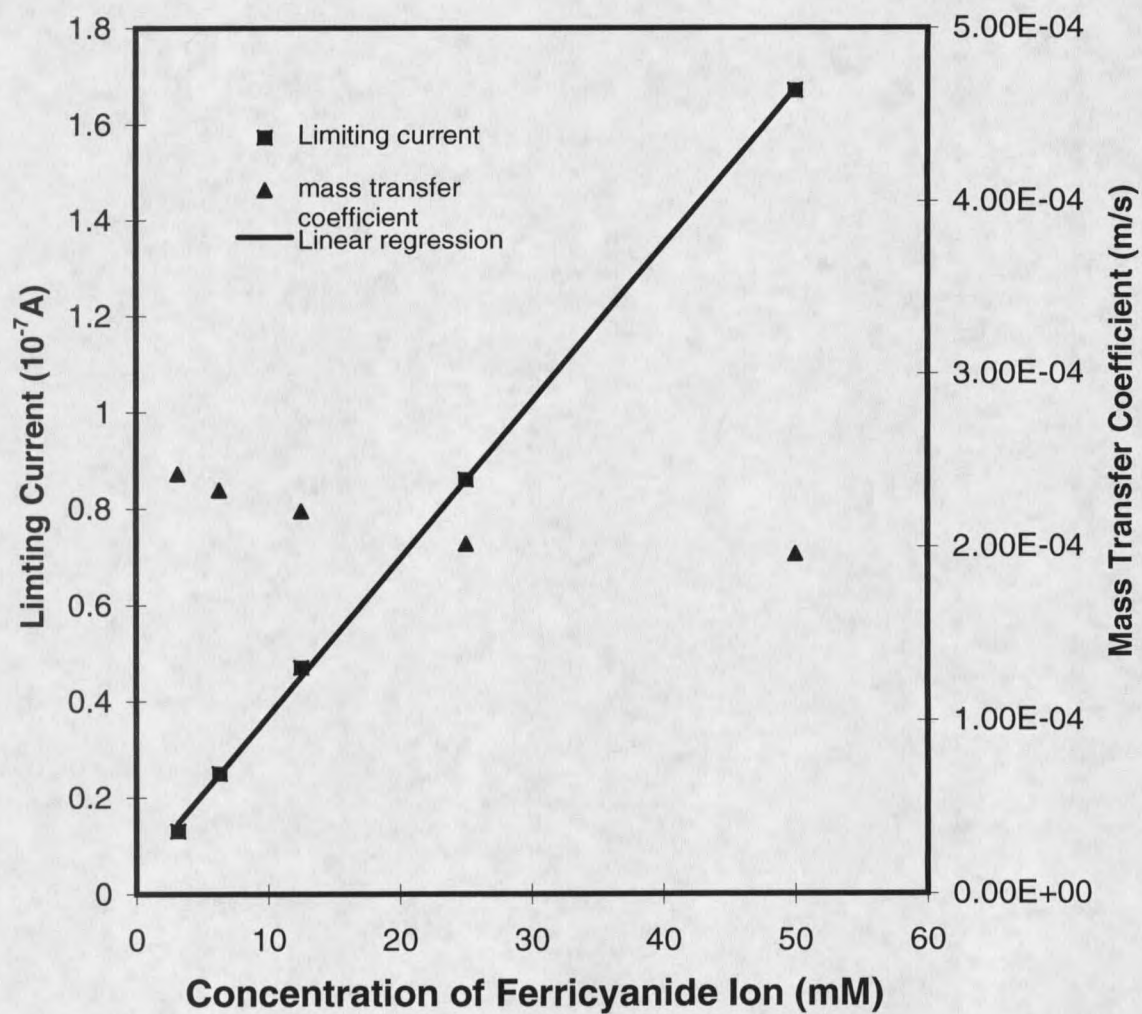


Fig. 5 Mass transfer coefficient vs . concentration of ferricyanide ion

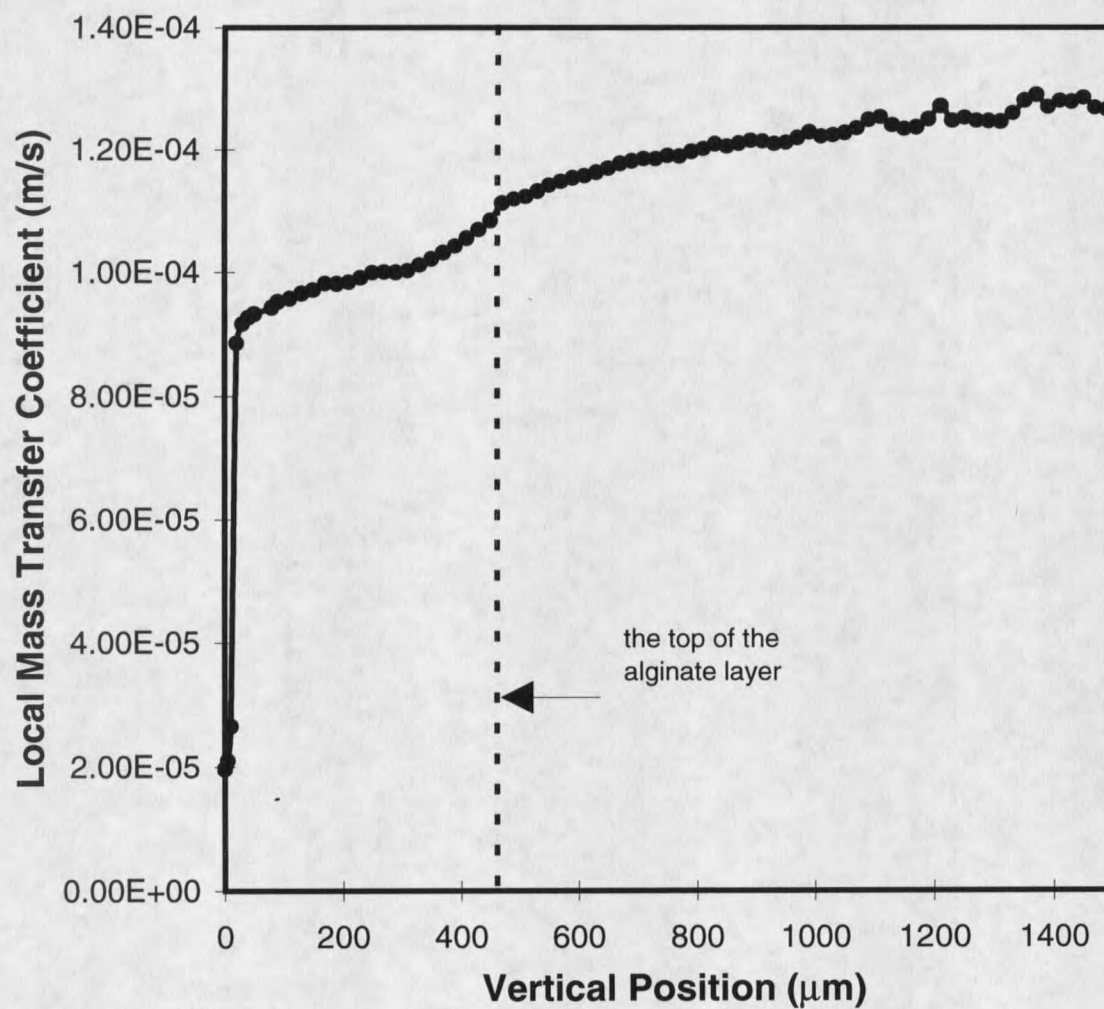


Fig. 6 Vertical profile of local mass transfer coefficient through an alginate layer

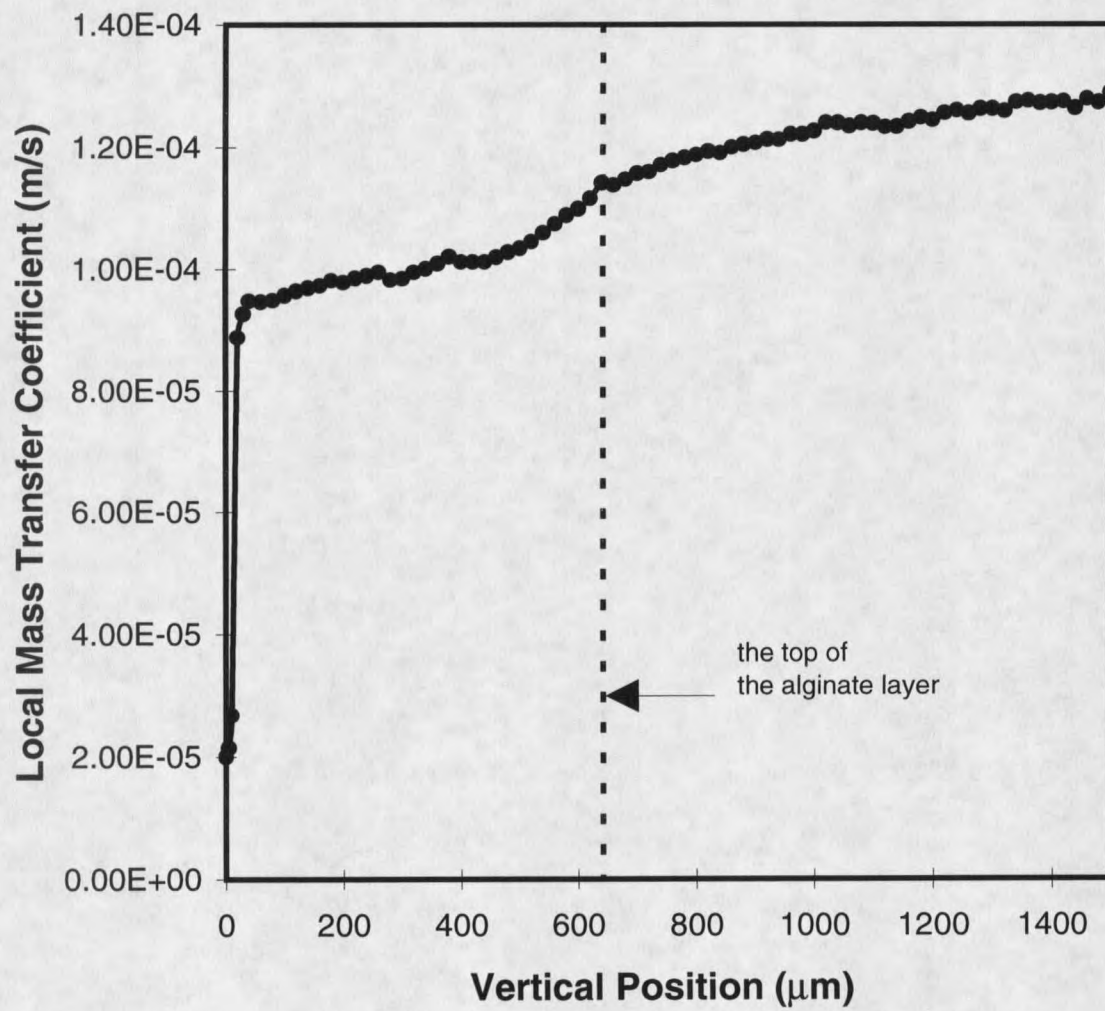


Fig. 7 Vertical profile of local mass transfer coefficient through an alginate layer

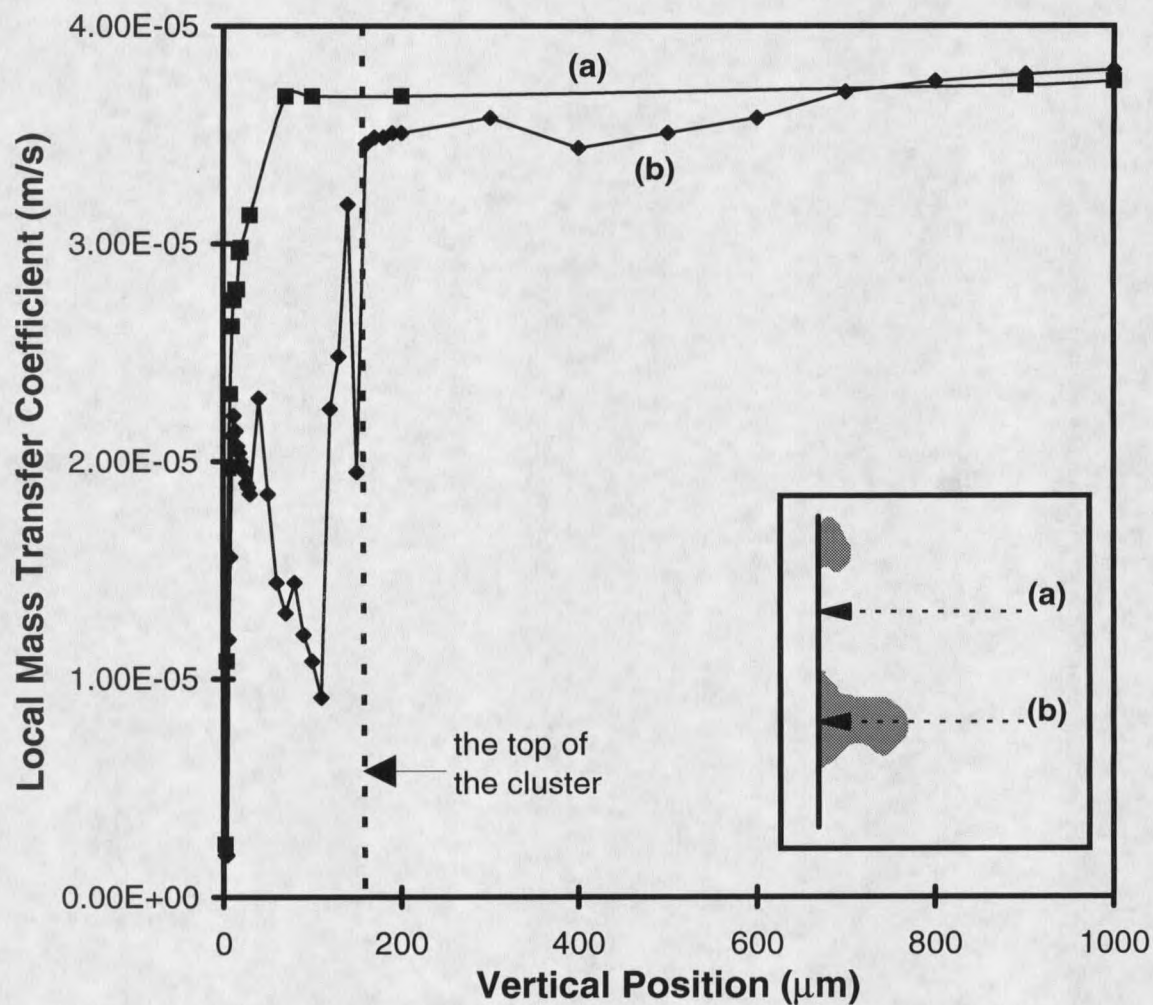


Fig. 8 Vertical profiles of local mass transfer coefficient measured in a void (a) and through a cluster (b). The insert is the schematic diagram that shows locations of measurement. The Shaded areas represent biofilm clusters.

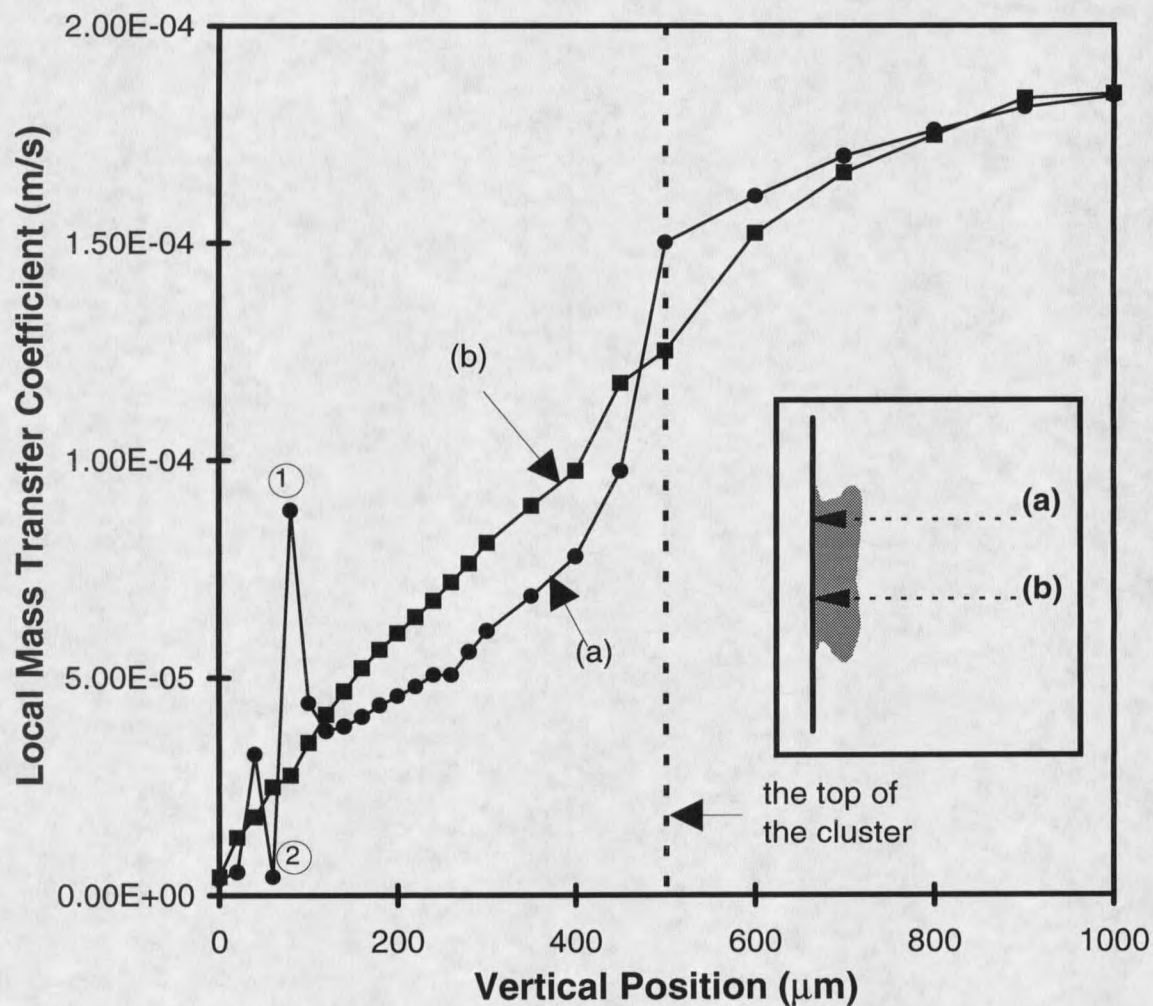
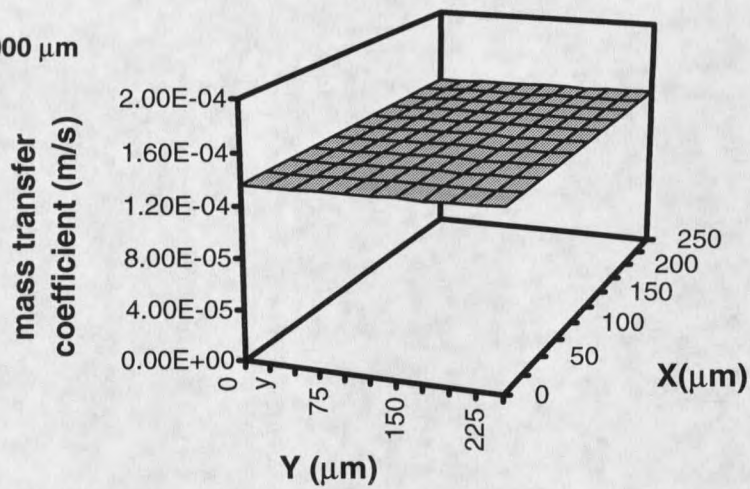
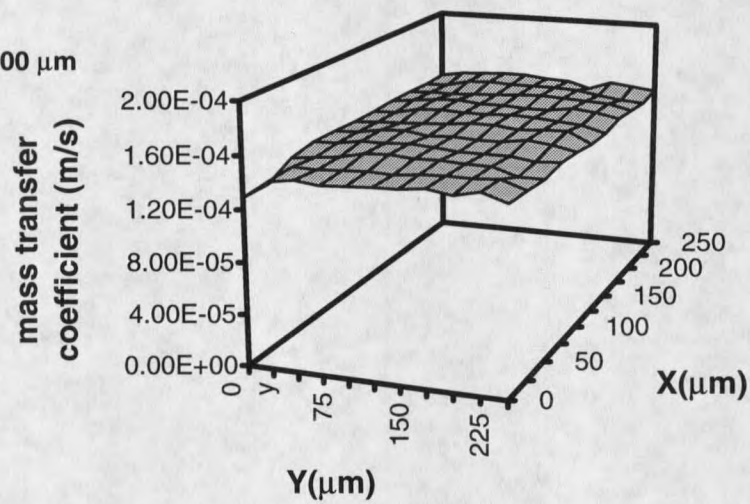
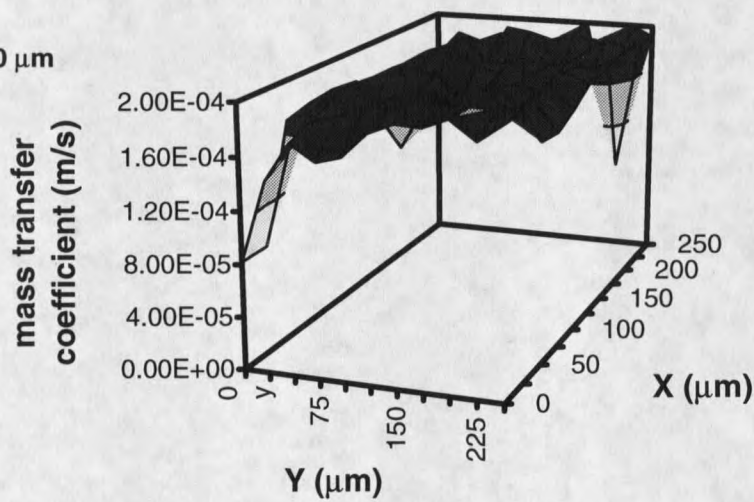


Fig. 9 Vertical profiles of local mass transfer coefficient measured at two different locations through a biofilm cluster. The insert is the schematic diagram that shows locations of measurement. The shaded area represents biofilm cluster.

(a) $z=1000\text{ }\mu\text{m}$ (b) $z=500\text{ }\mu\text{m}$ (c) $z=300\text{ }\mu\text{m}$ Fig. 10 Horizontal distributions of local mass transfer coefficient at $v=1.59\text{cm/sec}$

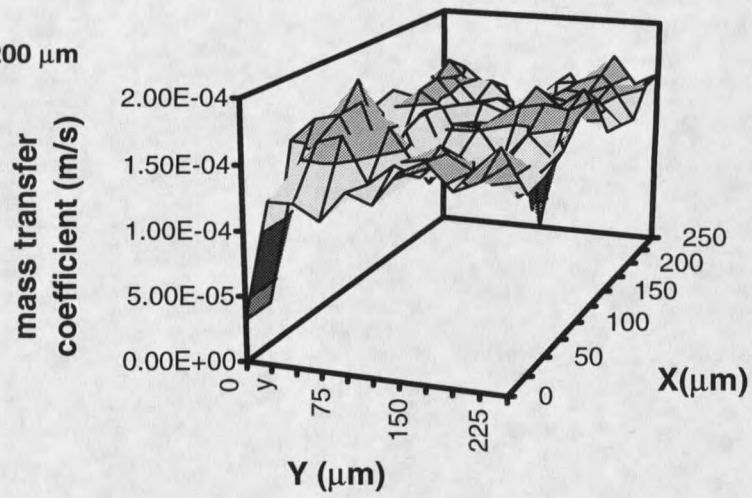
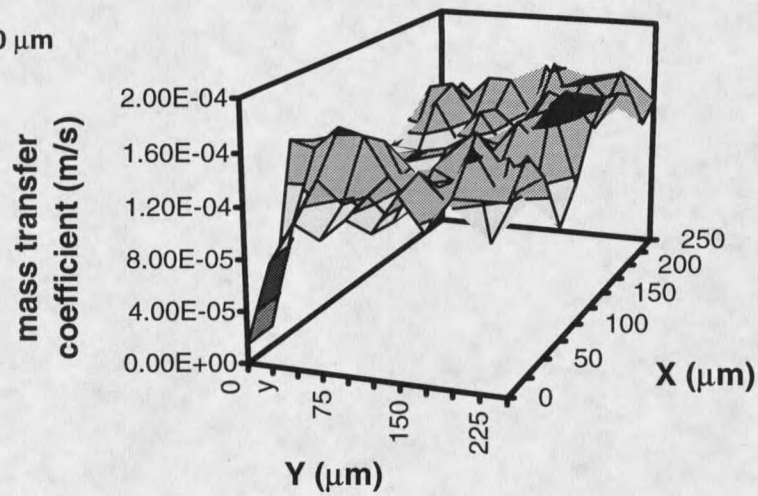
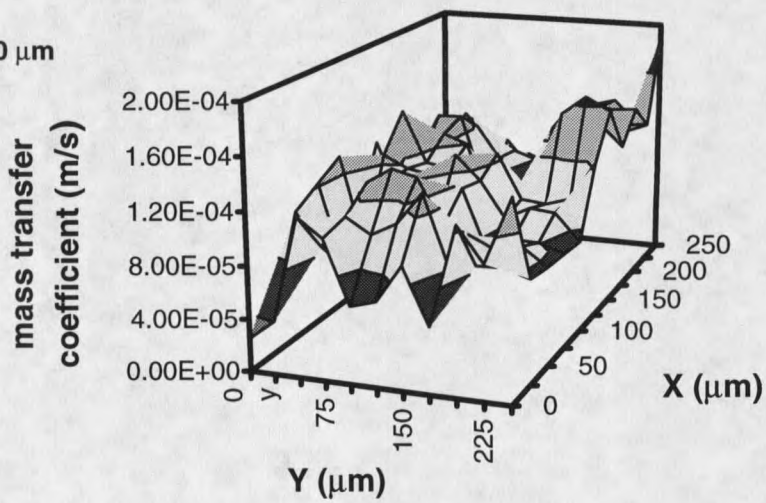
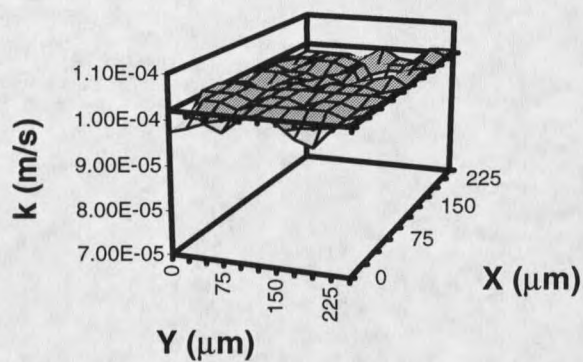
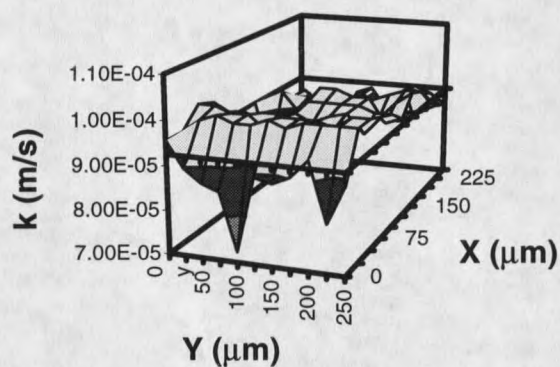
(d) $z=200\text{ }\mu\text{m}$ (e) $z=100\text{ }\mu\text{m}$ (f) $Z=50\text{ }\mu\text{m}$ 

Fig. 10 (continued)

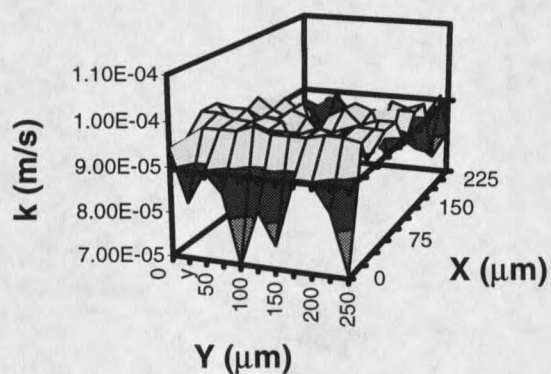
$V=1.40$ cm/sec



$V=0.88$ cm/sec



$V=0.76$ cm/sec



$V=0.41$ cm/sec

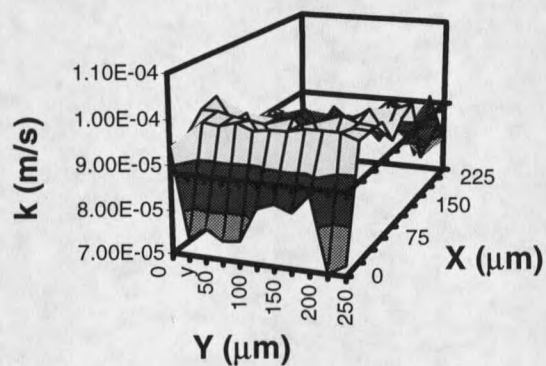


Fig. 11 Horizontal distributions of local mass transfer coefficient at $z=50$ μm.
The thickness of the biofilm cluster was 200 μm.

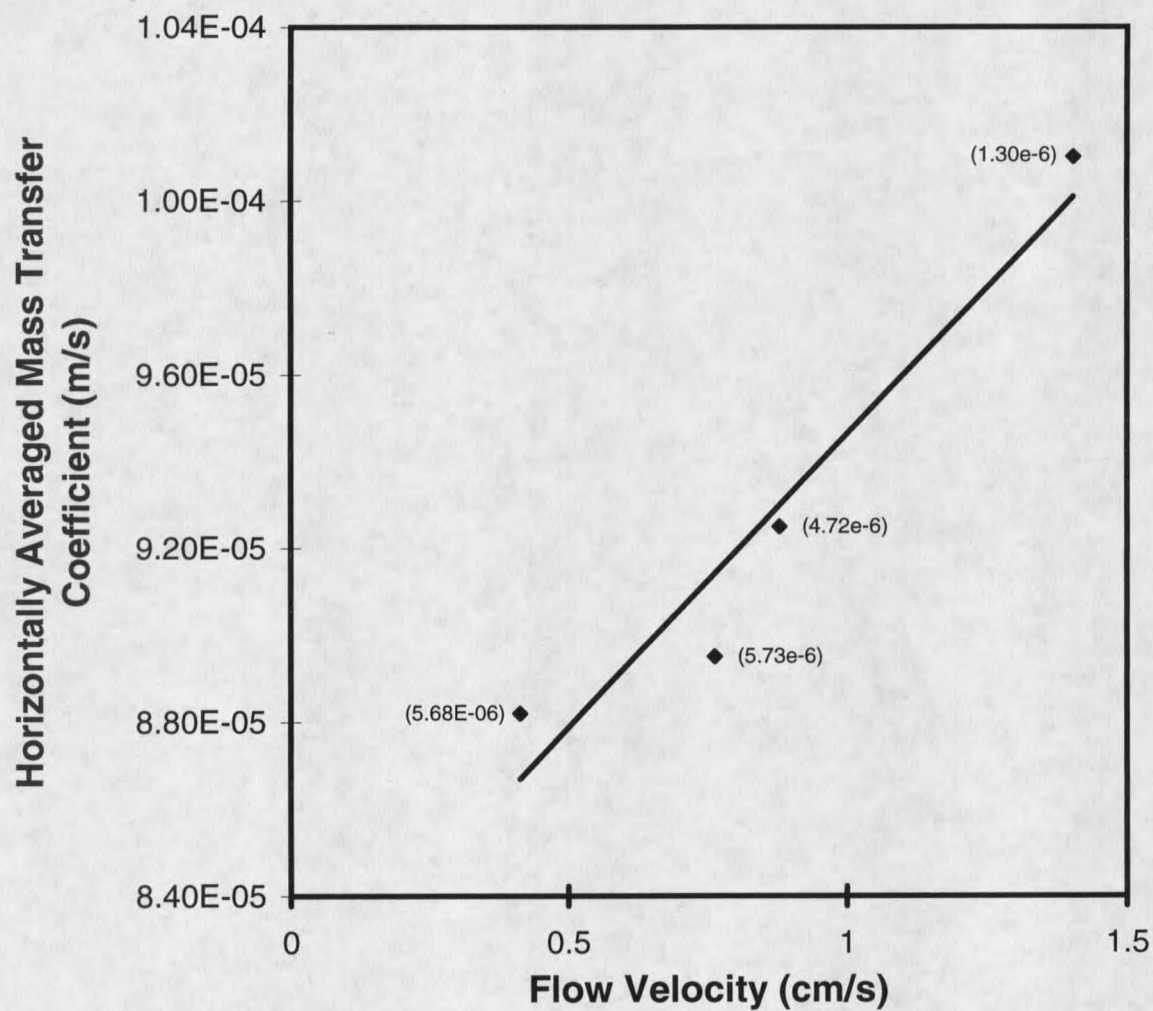


Fig. 12 Influence of bulk flow velocity on Horizontally averaged mass transfer coefficient at $z=50\text{ }\mu\text{m}$ for a $200\text{ }\mu\text{m}$ thick biofilm cluster. The values labeled next to data symbols are standard deviations of distributions

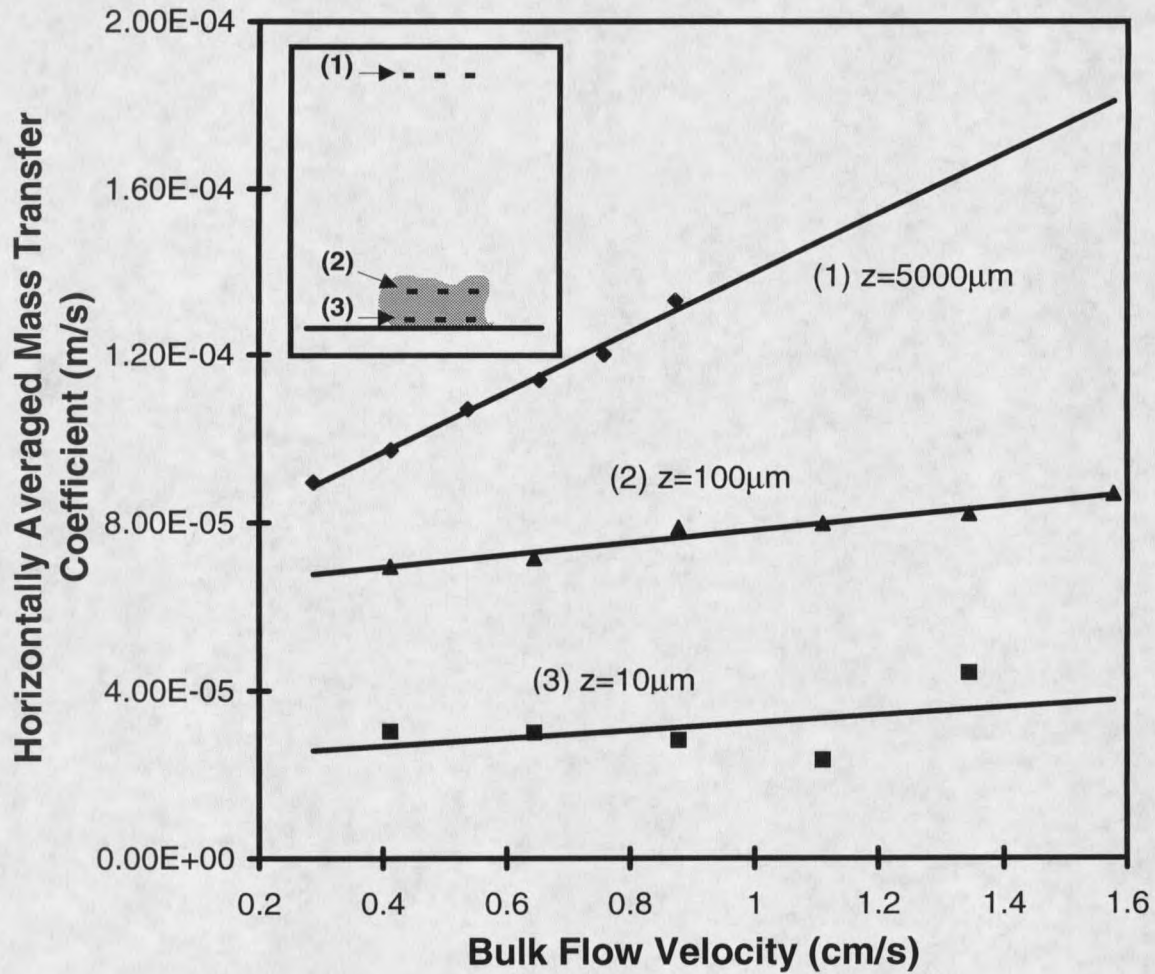


Fig. 13 Influence of bulk flow velocity on horizontally averaged mass transfer coefficient. As shown in the insert: (1) measured in bulk fluid at $z=5000\mu\text{m}$; (2) measured in biofilm at $z=100\mu\text{m}$; and (3) measured in biofilm at $z=10\mu\text{m}$. The shaded area represents biofilm cluster. Dashed lines show where horizontal distributions were taken from.

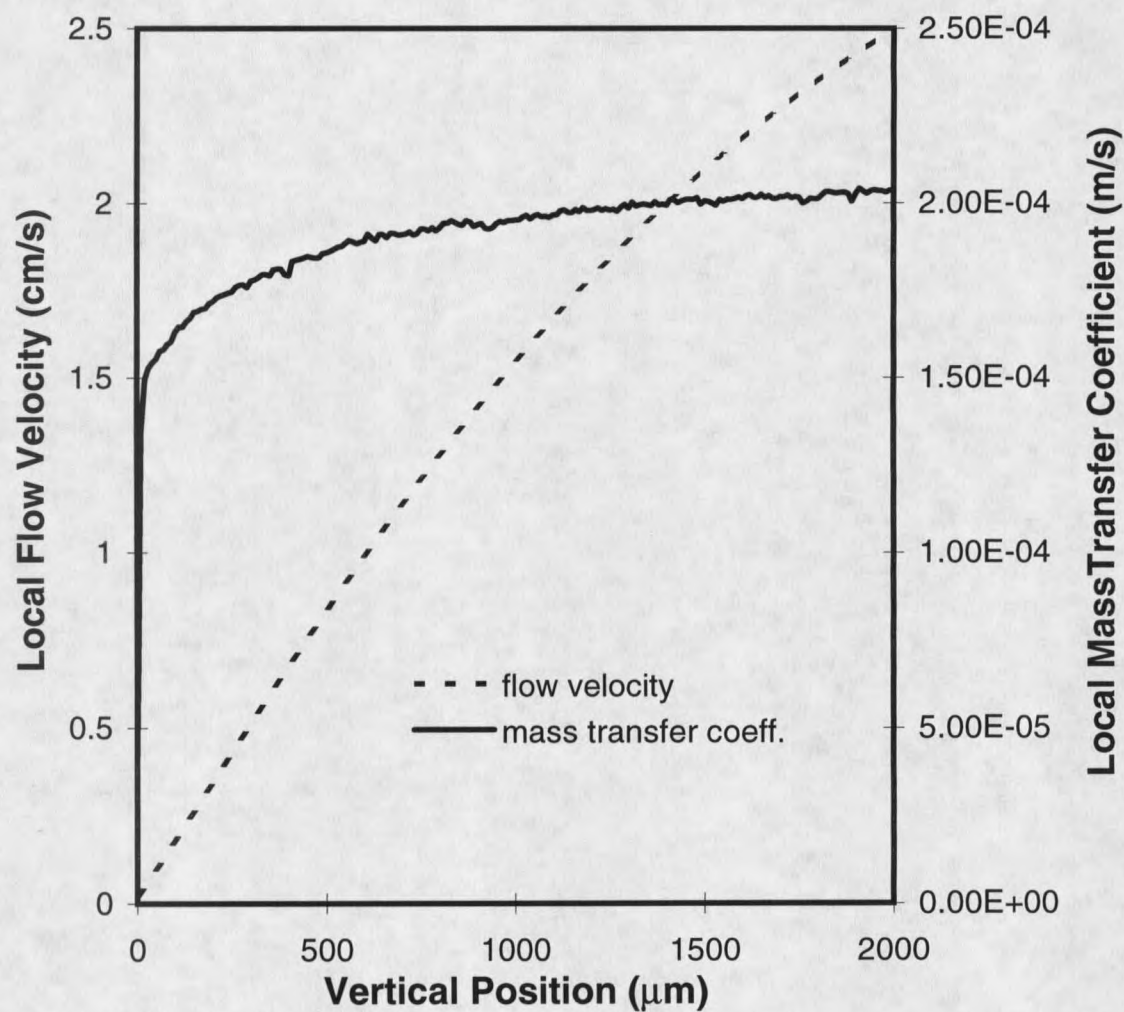


Fig. 14 Vertical profiles of local flow velocity and local mass transfer coefficient in a biofilm void

DISCUSSION

Experimental conditions influencing mass transfer limitation

To successfully apply LCT to mass transfer measurement, the overall electrochemical reaction occurring on the electrode tip, oxidation or reduction, has to be limited by mass transport. The most important experimental conditions are: (a) the surface condition of microelectrode, (b) the electrochemical reaction rate of reactive electrolyte. Both of them can be controlled experimentally. The surface condition of the microelectrode can be changed by polishing and cleaning and the electrochemical reaction rate is determined by properties of reactive electrolyte selected and electrochemical potential applied to the microelectrode.

Microelectrode cleaning

To ensure that the electrochemical reaction proceeds at the maximum possible rate and that the electrode response is reproducible, the surface of the electrode has to be prepared properly. In the literature³⁵, several cleaning procedures for large electrodes were proposed. The procedure mostly includes

acid rinsing and cathodic treating. We found these procedures unsatisfactory, results of voltammetry tests showed that electrodes prepared following these procedures did not provide a reasonable length of limiting current plateau. In many cases, electrodes showed a resistor-like behavior, i.e. measured current increased linearly as the applied potential increased. The mechanism of this behavior is believed to be due to the formation of a passive layer on the electrode surface. Instead of using reported cleaning procedures, a simple cleaning procedure involving sonication in water and acetone was described in the Methods and Materials. The method was found to be satisfactory and reasonable limiting current plateaus were achieved for microelectrodes.

Reacting electrolyte selection

The choice of electrochemical reactant for LCT applied in biofilm systems is narrowly restricted by the following criteria³⁵: (a) chemical stability; (b) high solubility; (3) low cost; (4) a reaction electrode potential that is sufficiently different from that of hydrogen evolution (or oxygen evolution) to give long, well-defined limiting current plateaus; and (5) less toxic or destructive to biofilm structure.

Commonly, an acidified copper sulfate solution is used from which copper is deposited on the electrode surface. However, because of the small surface area of the microelectrode such deposition will significantly alter the sensing area. An oxygen reduction system is also frequently used because of a long

limiting current plateau on platinum electrode surface. However, because oxygen is consumed by microorganisms in biofilm, local oxygen concentration can vary dramatically making it difficult to calculate mass transfer coefficient from measured limiting current according to Eqn. (6). An electrolyte system ($\text{Fe}(\text{CN})_6^{3-}$ / $\text{Fe}(\text{CN})_6^{4-}$) was considered to meet most of the listed criteria and was chosen to be used in biofilm mass transfer studies.

Applicability of LCT to biofilm system

The uniform distribution of $\text{Fe}(\text{CN})_6^{3-}$ concentration in biofilms suggests that $\text{Fe}(\text{CN})_6^{3-}$ either does not react with biofilms or reacts very slowly. Since n , A , F , and C_0 are all constants, the mass transfer coefficient only depends on limiting current according to Eqn. (6) and can be calculated easily.

Fig. 5 presents the linear relation between the measured limiting current and $\text{Fe}(\text{CN})_6^{3-}$ concentration employed. Mass transfer coefficients were calculated for different ferricyanide concentrations. The average mass transfer coefficient was $2.19 \times 10^{-4} \pm 0.20 \times 10^{-4}$ m/s. Because the standard deviation was small (<10%), the measured mass transfer coefficient is assumed independent of the concentration of reacting species.

The major concern of applying LCT to determine local mass transfer coefficients in biofilm systems was whether the biofilm structure would remain

unchanged during the measurement. The time required to complete a set of measurements ranged from 2 to 10 hours. 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 many biofilm microorganisms remained viable, despite the harsh conditions during the experiments. Since 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 was applicable to biofilm systems and did not alter mass transfer process in biofilms.

Estimation of measurement errors on mass transfer coefficient

Since the mass transfer coefficient is calculated according to Eqn.(6), the possible measurement error for mass transfer coefficient mainly comes from errors in measuring A , C_0 , and I .

Possible error in measuring sensing area of microelectrode

The diameter of the microelectrode was measured under a microscope with a 100 $\times$ magnification lens. For a 10 μm diameter microelectrode, the possible measurement error was estimated to be $\pm 0.2 \mu\text{m}$ or 2% of measurement error in diameter which translated into a $\pm 4\%$ error for the sensing area estimation.

Possible error in measuring bulk concentration of $\text{Fe}(\text{CN})_6^{3-}$

The possible error in measuring C_0 by the titration method can be estimated as follows: a typical 25 ml of 25 mM $\text{Fe}(\text{CN})_6^{3-}$ solution consumes 6.25 ml of 0.1 M $\text{Na}_2\text{S}_2\text{O}_3$ titrant. During the titration, the possible titration error was ± 2 drops of titrant which was measured to be 0.14 ml of the titrant volume. The error of C_0 measurement was then estimated to be $\pm 2\%$ ($=0.14 \text{ ml}/6.25 \text{ ml}$).

Possible error in measuring limiting current

The limiting current was measured using HP-4140 multimeter whose measuring accuracy in current is at the level of 10^{-12} Ampere. The typical current value measured in this study was in the range of 10^{-7} to 10^{-9} Ampere. Therefore, the error in measuring current was negligible.

Other possible error

Besides measurement errors discussed above, another source of error comes from the contribution of electromigration to the limiting current. The migration contribution provides a positive error because the existence of migration process will increase the limiting current. For $\text{Fe}(\text{CN})_6^{3-} / \text{Fe}(\text{CN})_6^{4-}$ system, it is estimated³⁵ that the migration contribution is less than 1% with

the addition of excess amount of non-reacting electrolyte, KCl, to the electrolyte solution.

By combining all possible error sources, the maximum measurement error for the obtained mass transfer coefficient was estimated to be between + 7% and -5%.

Influence of convective flow velocity on mass transfer coefficient

In these studies, the flow velocities used ranged from 0.2 to 1.6 cm/sec and the corresponding range of Reynolds numbers ($Re=4 \times V \times R_H / \nu$) was 50 to 500 where R_H is the hydraulic radius of the rectangular flow channel. Therefore, results and conclusions reported here are only applicable to these flow conditions.

Horizontal distributions presented in Fig. 11 show that bulk flow velocity does not just influence the average mass transfer coefficient, as shown in Fig. 12, but also influences the fluctuation of the local mass transfer coefficient. By performing a simple statistical analysis on all four distributions, we can see that the standard deviation of local mass transfer coefficients (numbers labeled next to average data points in Fig. 12), decreased as the flow velocity increased.

In the bulk flow, convective transport is the dominant process of mass transport. From the results presented in Curve (1) of Fig. 13, 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;

$$k(m/s) = 7.10 \times 10^{-3} \times V(m/s) + 6.83 \times 10^{-3} \quad (14)$$

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 relationship between average mass transfer coefficient and bulk flow velocity also holds reasonably well inside the biofilm, as shown in Curve (2) & (3) of Fig. 13. 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 bulk fluid nutrients are more accessible in the top portion of biofilms than in the bottom, even though the liquid flows inside the entire biofilm. The intercepts of the curves are the mass transfer coefficients at zero flow velocity.

Analysis of horizontal distribution of local mass transfer coefficient

The results presented in Fig. 10 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 Fig. 10 are given in Table 2. Significant fluctuations of mass transfer coefficient occurred just above the top of the solid biofilm matrix, $z=200$ μm , and inside the biofilm, but not above a water channel.

Table 2. analysis of horizontal distributions in Fig. 10

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

For the flow velocity of 1.58 cm/s, in the bulk flow at $z=1000$ μm , 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 μm , 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 vs. z in Fig. 15. A separate experimental result, presented in Fig. 16, shows that what happened above the top of biofilm clusters was not an isolated phenomenon. These changes suggest the existence of increased mixing in this region. Turbulence could be the result of the interaction between structure of the

biofilm matrix and fluid hydrodynamics. Bioturbation^{28,36} (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, as is commonly believed, the local mass transfer coefficient should be relatively constant. However, the horizontal distributions presented in Fig. 10e and 10f show that local mass transfer coefficient inside the biofilm was 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 Fig. 8 and 9. The sudden increases of the mass transfer coefficient in Curve (b) of Fig. 8 and Curve (a) of Fig. 9 show that 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 as presented in Fig. 6 and 7. 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 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, the change in convective flow velocity was estimated for the observed fluctuation of local mass transfer coefficient. The difference of mass transfer coefficients, Δk , between point ① and ② in Curve (a) of Fig. 9 is 8×10^{-5} m/sec. According to the slope of Eqn. 14, the change in flow velocity, ΔV , is calculated to be 1.13 cm/sec. 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 Fig. 17. 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 possibility of flow channels inside biofilm clusters adds an additional dimension of heterogeneity to the existing biofilm model. This extended model contributes to our understanding of biofilm heterogeneity and may also help explain some puzzling experimental results, such as the one reported by Jansen and Kristensen¹⁵ who measured a higher diffusivity in biofilm than that in pure

water. The results are not surprising if convective transport inside biofilm is considered.

Local shear rate on microelectrode surface

As discussed in the Theory chapter, the effect of the local shear rate on the microelectrode surface can be calculated from the local mass transfer coefficient according to Eqn. (13). The equation is only valid when a circular-disk microelectrode is physically present in a liquid flow field. Since results presented in Curve (1) of Fig. 12 were taken in bulk flow, corresponding local shear rates were calculated according to Eqn. (13) and are shown in Fig. 18. By curve fitting, the relation between local shear rate on the circular-disk microelectrode and bulk flow velocity can be expressed in a simple exponential formula

$$S = -1.006 + 0.3522 \times e^{4.159 \times V} \quad (15)$$

The relation indicates that local shear rate on a rigid microelectrode surface increases exponentially with the increase of bulk flow velocity in the studied velocity range. Although it is unknown whether the conclusion can be applied to viscoelastic biofilm surfaces, the technique enables us for the first time to study spatial changes in local shear stress.

To correlate biofilm detachment with the flow rate and shear rate in specific biofilm reactors, further study is suggested that microelectrodes can be

introduced into the biofilm from below in order to substitute biofilm surface with microelectrode surface.

Correlation of local flow velocity and local mass transfer coefficient

It has been demonstrated that LCT can be combined with other techniques, such as CSLM, to obtain additional information. Fig. 14 shows the quantitative relation between local mass transfer coefficient and local flow velocity measured at the same location in a biofilm void. By plotting local mass transfer coefficient vs. local flow velocities as shown in Fig. 19, a simple logarithmic relationship is revealed except for the last data point which was taken at the bottom of the reactor.

It should be pointed out that only the horizontal component of flow velocities is measured by PIV; three dimensional flow velocities cannot yet be measured with this technique. Above biofilm clusters, the possible turbulent flow as discussed earlier makes it impossible to use PIV to measure local flow velocity and correlate it with local mass transfer coefficients.

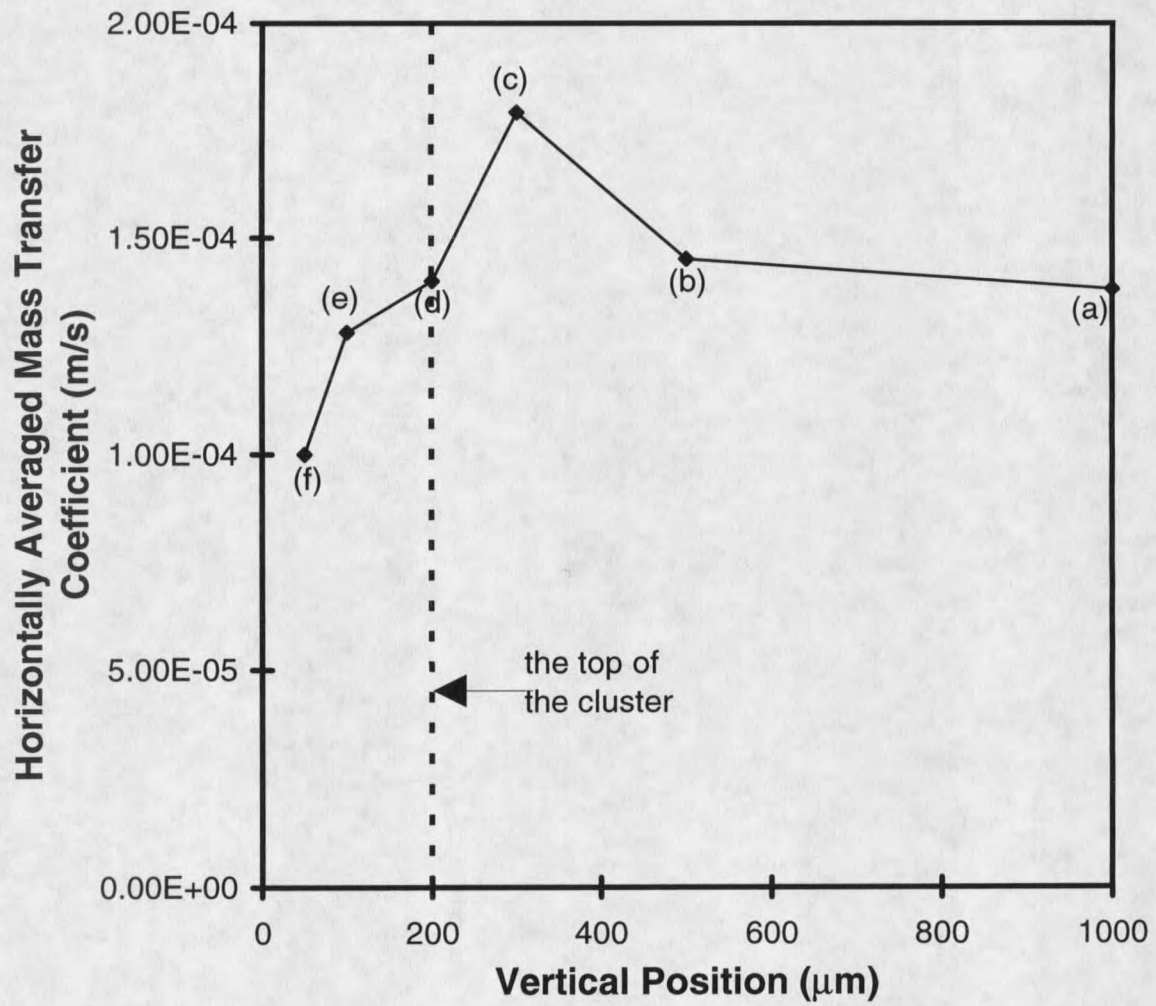


Fig. 15 Vertical profile of horizontally averaged mass transfer coefficient calculated from Fig. 10

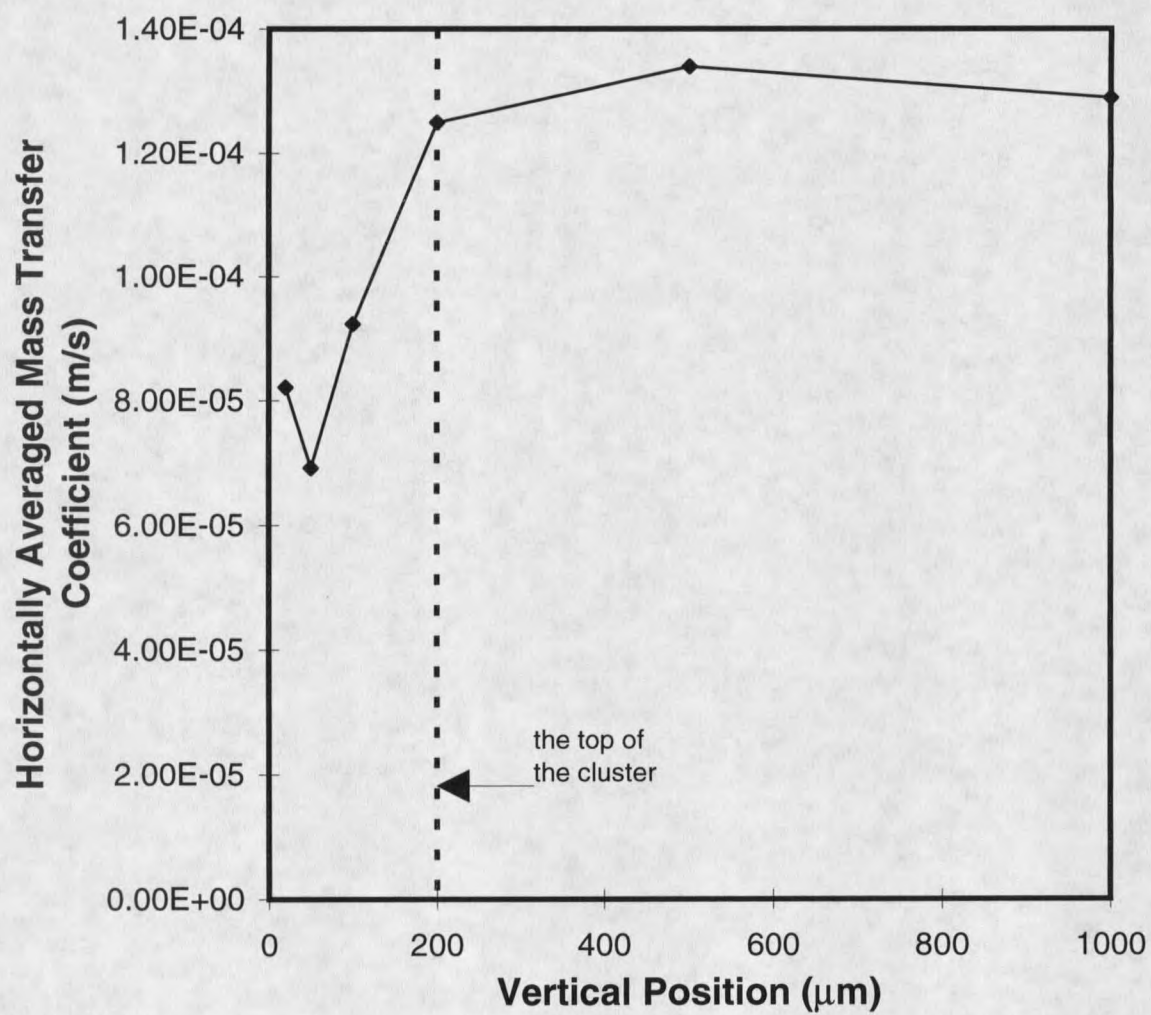


Fig. 16 Vertical profile of horizontally averaged mass transfer coefficient for a 200 μm thick biofilm cluster. Flow velocity=0.75 cm/sec

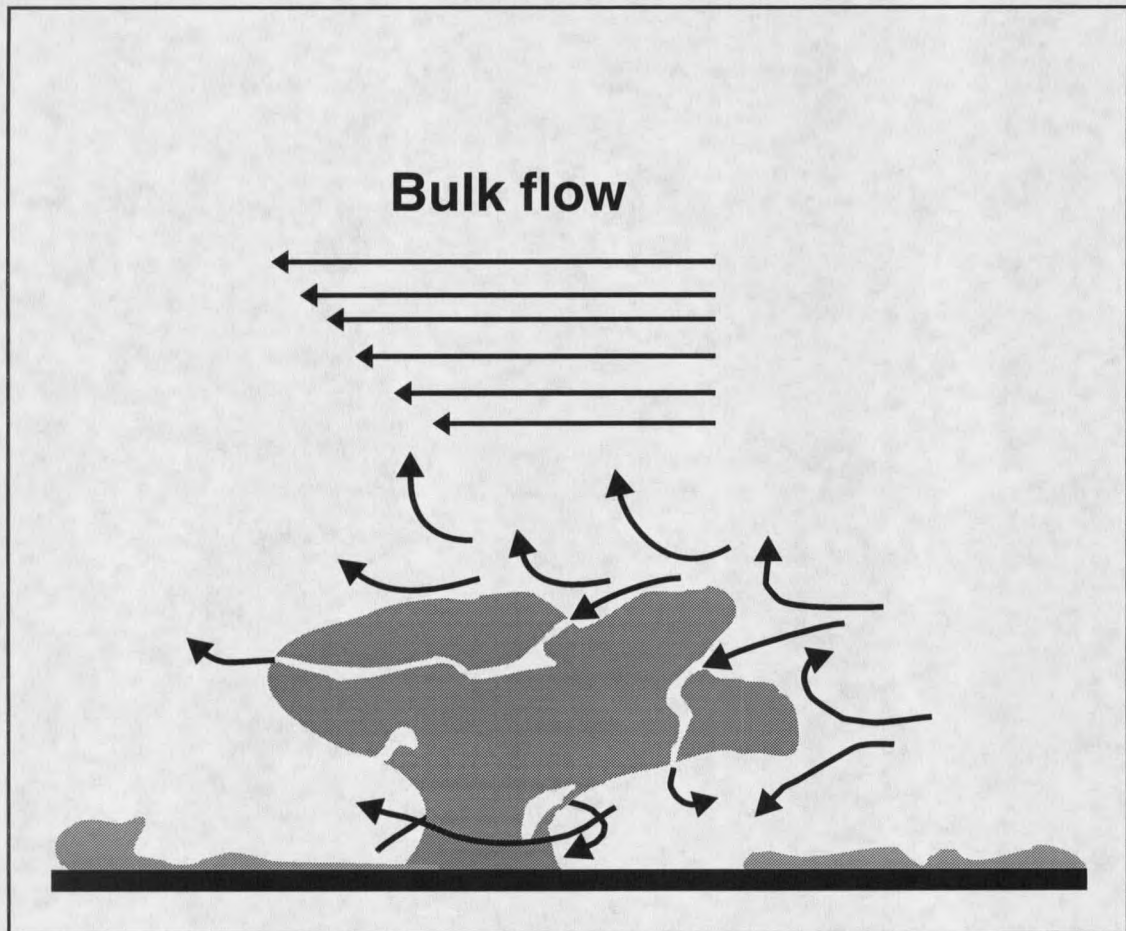


Fig. 17 The proposed structural model of biofilm cell cluster

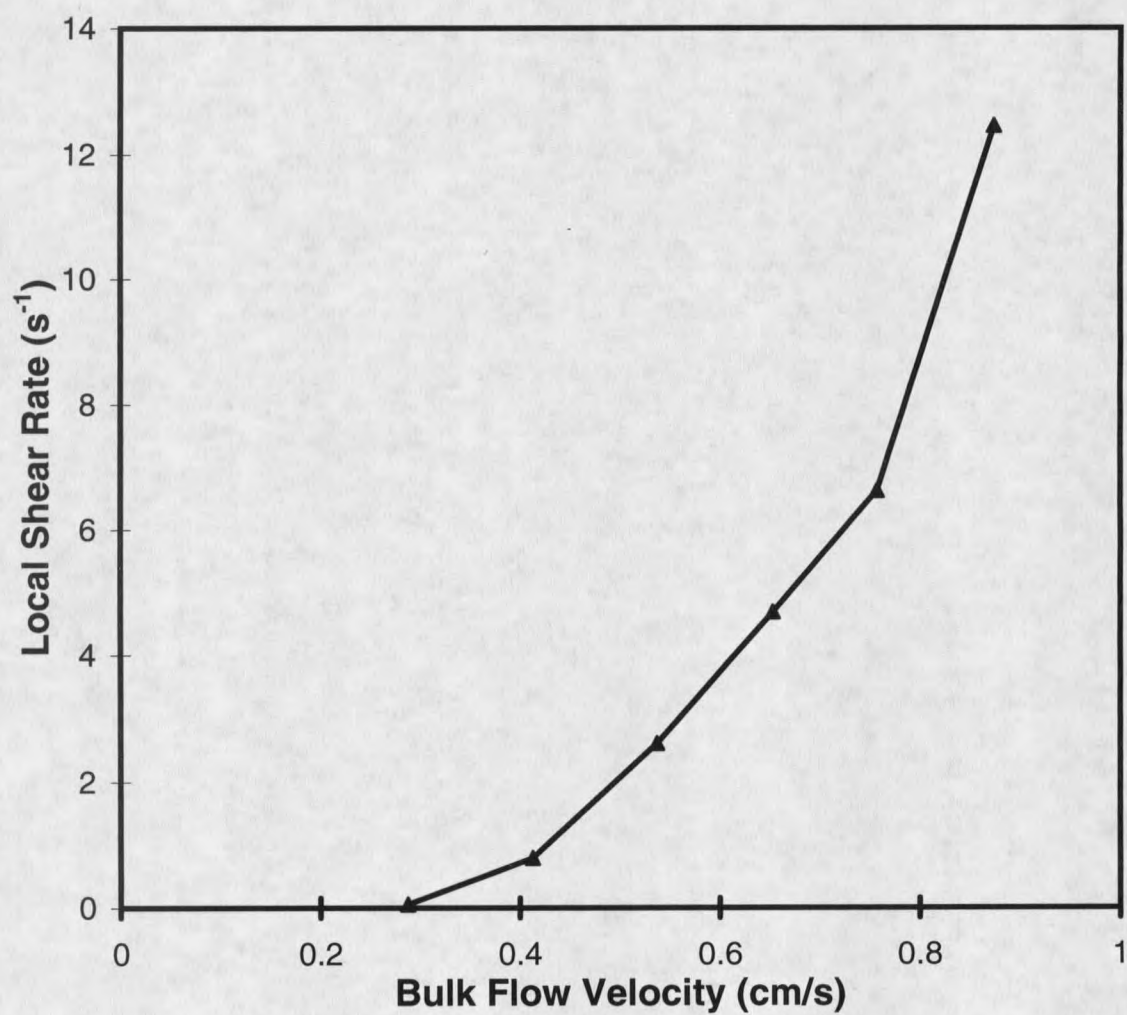


Fig. 18 The relationship between local shear rate on circular disk microelectrode and bulk flow velocity

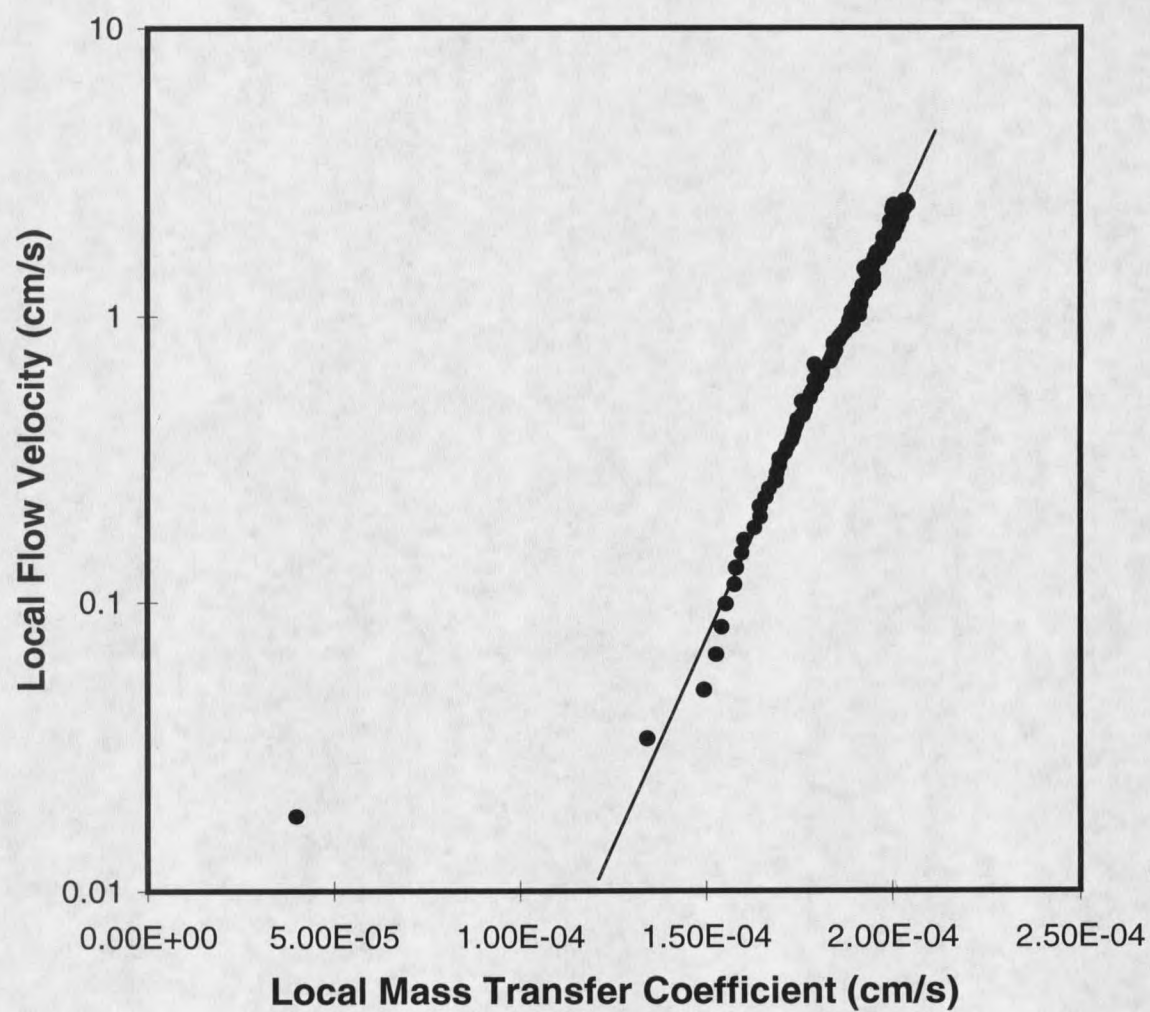


Fig. 19 Correlation between local mass transfer coefficient and local flow velocity in a biofilm void

SUMMARY

Technique development and applications

An experimental technique has been developed for measuring local mass transfer coefficients in biofilms. The technique was adapted from a conventional limiting current technique. The electrode used was mobile and its diameter was reduced to a few micrometers to prevent damaging the biofilm structure and disturbing the hydrodynamics. The tip of the microelectrode formed a microsink for an electroactive species purposely added to the reactor. The local mass transfer coefficient calculated from the measured limiting current to the microelectrode is related to the local effective diffusivity which is a function of local hydrodynamics which in turn depends on local biofilm structure. Viable plate counts and time-lapse microscopy showed that biofilm structure remained intact and biofilm remained viable during limiting current measurements. The overall measurement error for local mass transfer coefficient was estimated to be between +7% to -5%.

This thesis also explored the possible application of using LCT to measure local shear stress in biofilm reactor. A flow field model was developed for the

circular-disk microelectrodes. Analytical relationships between mass transfer coefficient and shear rate were established. The calculated local shear rate data showed the exponential increase with the increase of bulk flow velocity.

Mass transfer process in aerobic biofilms

By applying LCT to biofilm systems, vertical profiles and horizontal distributions of the local mass transfer coefficient in biofilms were obtained. The following conclusions can be drawn from these results:

1. The influence of hydrodynamics on the average mass transfer coefficient decreases into the biofilm away from the bulk fluid.

2. The mass transfer coefficient increases just above the biofilm surface. This suggests the existence of turbulence in the immediate region just above the biofilm.

3. Vertical profiles of the local mass transfer coefficient measured across interstitial voids are similar to those measured in the absence of biofilm.

4. Vertical profiles of the local mass transfer coefficient measured across biofilm matrixes demonstrated unexplained variability. This variability was attributed to the heterogeneous structure of biofilm matrix.

RECOMMENDATIONS FOR FUTURE RESEARCH

1. The technique presented in this thesis should be further applied for the quantification of local mass transfer rates in other biofilm systems to gain a broader and more general understanding of the mass transfer process in heterogeneous biofilms.
2. The technique has shown its potential application for study of local shear rate in biofilm system. More investigation is needed to understand local shear rate information and relationships between biofilm detachment into biofilm or sloughing and local shear stress. It is suggested that a microelectrode be introduced from bottom of reactor so that microelectrode surface can experience the same flow condition that the biofilm surface does.
3. The technique also has potential for investigating local turbulent intensity and frequency in biofilms due to its rapid response characteristics.
4. In order to use the measured local mass transfer coefficients in existing biofilm models, the theoretical relationship between apparent diffusivity and local mass transfer coefficient to the microelectrode has to be established.
5. For the case of no flow, LCT may be modified to perform Potential Step Chronoamperometry to directly measure effective diffusivity inside biofilm.

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NOMENCLATURE

[Units: M--mass, L--length, T--time]

A is the sensing area of microelectrode, (L^2).

C is the concentration of substrate, (ML^{-3}).

C_o is the concentration of reacting electrolyte in the bulk, (ML^{-3}).

C_F is the concentration of substrate on biofilm surface, (ML^{-3}).

C_s is the concentration of reacting electrolyte on electrode surface, (ML^{-3})

C_x is the concentration of biomass, (ML^{-3}).

D is the diffusivity of substrate, (L^2T^{-1})

F is the Faraday's constant, =96485 C/mole

I is the electrical current, (Ampere)

J is the flux of reactant, ($MT^{-1}L^{-2}$)

k is the mass transfer coefficient, (LT^{-1})

K_c is the saturation constant, (ML^{-3}).

n is the number of mole of electron transferred, (M)

Pe is Peclet number (= $S \times r^2 / D$)

R_H is the hydraulic radius of the reactor, (L)

Re is Reynolds number, (= $V \times r / \nu$)

r is the radius of microelectrode, (L)

S is the shear rate, (T^{-1})

Sh is Sherwood number, (= $k \times r / D$)

V is the flow velocity, (LT^{-1})

x is the direction of liquid flow, (L)

z is the vertical position perpendicular to bottom of biofilm reactor, (L)

ΔC is the concentration difference, (ML^{-3}).

δ is the thickness of external mass transfer boundary layer, (L).

$$\eta = z/r$$

$$\theta = C/C_o$$

$$\xi = x/r$$

$$\phi = (x^2 + y^2)^{1/2}$$

τ is the shear stress, ($=\mu \times S$)

μ is the viscosity of electrolyte solution, (cp)

ρ is the density of electrolyte solution, (ML^{-3})

ν is the kinematic viscosity, ($=\mu/\rho$)

APPENDIX

Experimental Raw Data

Raw data of Fig. 5

Fe(CN) ₆ ³⁻ conc. (mM)	Limiting current ($\times 10^7$ A)	Mass transfer coefficient (m/s)
3.15	0.13	2.42×10^{-4}
6.3	0.25	2.33×10^{-4}
12.5	0.47	2.21×10^{-4}
25	0.86	2.02×10^{-4}
50	1.67	1.96×10^{-4}

Diameter of microelectrode: 15 μm

Flow velocity: 1.83 cm/sec

Raw data of Fig. 6

Verical Position (μm)	limiting current ($\times 10^7$ A)	k (m/s)	Verical Position (μm)	limiting current ($\times 10^7$ A)	k (m/s)	Verical Position (μm)	limiting current ($\times 10^7$ A)	k (m/s)
0	0.49624	0.00012	620	0.49116	0.00012	1240	0.43096	0.00011
20	0.50093	0.00013	640	0.48814	0.00012	1260	0.42505	0.00011
40	0.49815	0.00013	660	0.48667	0.00012	1280	0.41948	0.00011
60	0.50025	0.00013	680	0.48584	0.00012	1300	0.41441	0.0001
80	0.50293	0.00013	700	0.48853	0.00012	1320	0.41006	0.0001
100	0.51255	0.00013	720	0.48496	0.00012	1340	0.40606	0.0001
120	0.50943	0.00013	740	0.48184	0.00012	1360	0.40225	0.0001
140	0.50049	0.00013	760	0.4811	0.00012	1380	0.39849	0.0001
160	0.49668	0.00012	780	0.48262	0.00012	1400	0.39741	1E-04
180	0.50181	0.00013	800	0.48306	0.00012	1420	0.39775	0.0001
200	0.50337	0.00013	820	0.48115	0.00012	1440	0.39717	1E-04
220	0.50474	0.00013	840	0.47935	0.00012	1460	0.39409	9.9E-05
240	0.51123	0.00013	860	0.48071	0.00012	1480	0.39121	9.8E-05
260	0.5086	0.00013	880	0.47774	0.00012	1500	0.39009	9.8E-05
280	0.50918	0.00013	900	0.47598	0.00012	1520	0.39033	9.8E-05
300	0.50513	0.00013	920	0.4729	0.00012	1540	0.38623	9.7E-05
320	0.51314	0.00013	940	0.47329	0.00012	1560	0.38399	9.7E-05
340	0.50943	0.00013	960	0.47124	0.00012	1580	0.38071	9.6E-05
360	0.50103	0.00013	980	0.47129	0.00012	1600	0.37861	9.5E-05
380	0.49561	0.00012	1000	0.46978	0.00012	1610	0.375	9.4E-05
400	0.49585	0.00012	1020	0.46812	0.00012	1640	0.3709	9.3E-05
420	0.49614	0.00012	1040	0.46504	0.00012	1650	0.3687	9.3E-05
440	0.4981	0.00013	1060	0.46245	0.00012	1660	0.3646	9.2E-05
460	0.49619	0.00012	1080	0.46016	0.00012	1670	0.35234	8.9E-05
480	0.50567	0.00013	1100	0.45864	0.00012	1680	0.10537	2.6E-05
500	0.49717	0.00013	1120	0.4564	0.00011	1685	0.08335	2.1E-05
520	0.49185	0.00012	1140	0.45396	0.00011	1690	0.07803	2E-05
540	0.49087	0.00012	1160	0.45015	0.00011			
560	0.49326	0.00012	1180	0.44668	0.00011			
580	0.49863	0.00013	1200	0.44512	0.00011			
600	0.49673	0.00012	1220	0.44243	0.00011			

Diameter of the microelectrode: 5 μm

Flow velocity: 1.15 cm/sec

Fe(CN)₆³⁻ conc.: 21 mM

Raw data of Fig. 7

Verical Position (μm)	limiting current ($\times 10^7 \text{ A}$)	k (m/s)	Verical Position (μm)	limiting current ($\times 10^7 \text{ A}$)	k (m/s)	Verical Position (μm)	limiting current ($\times 10^7 \text{ A}$)	k (m/s)
0	0.50278	0.00013	580	0.49019	0.00012	1180	0.41543	0.0001
20	0.50703	0.00013	600	0.49248	0.00012	1200	0.41104	0.0001
40	0.50567	0.00013	620	0.49292	0.00012	1220	0.4085	0.0001
60	0.50425	0.00013	640	0.49077	0.00012	1240	0.40532	0.0001
80	0.50205	0.00013	660	0.49282	0.00012	1260	0.40225	0.0001
100	0.50254	0.00013	680	0.49317	0.00012	1280	0.40225	0.0001
120	0.50532	0.00013	700	0.48745	0.00012	1300	0.4021	0.0001
140	0.50552	0.00013	720	0.4855	0.00012	1320	0.40571	0.0001
160	0.50957	0.00013	740	0.4855	0.00012	1340	0.40108	0.0001
180	0.51563	0.00013	760	0.48203	0.00012	1360	0.39775	0.0001
200	0.51235	0.00013	780	0.48223	0.00012	1380	0.39561	9.9E-05
220	0.5061	0.00013	800	0.47984	0.00012	1400	0.3915	9.8E-05
240	0.50845	0.00013	820	0.47891	0.00012	1420	0.39053	9.8E-05
260	0.50249	0.00013	840	0.47691	0.00012	1440	0.39556	9.9E-05
280	0.50684	0.00013	860	0.47324	0.00012	1460	0.39331	9.9E-05
300	0.50606	0.00013	880	0.47427	0.00012	1480	0.39165	9.8E-05
320	0.50567	0.00013	900	0.47207	0.00012	1500	0.38906	9.8E-05
340	0.50718	0.00013	920	0.47012	0.00012	1520	0.39024	9.8E-05
360	0.50625	0.00013	940	0.46812	0.00012	1540	0.38696	9.7E-05
380	0.50064	0.00013	960	0.46548	0.00012	1560	0.38535	9.7E-05
400	0.502	0.00013	980	0.46094	0.00012	1580	0.3833	9.6E-05
420	0.50205	0.00013	1000	0.45986	0.00012	1600	0.38047	9.6E-05
440	0.49902	0.00013	1020	0.45596	0.00011	1620	0.3773	9.5E-05
460	0.50093	0.00013	1040	0.45225	0.00011	1640	0.37632	9.5E-05
480	0.49927	0.00013	1060	0.45361	0.00011	1660	0.37691	9.5E-05
500	0.49468	0.00012	1080	0.4436	0.00011	1670	0.36856	9.3E-05
520	0.49644	0.00012	1100	0.43672	0.00011	1680	0.35293	8.9E-05
540	0.49365	0.00012	1120	0.43247	0.00011	1690	0.10669	2.7E-05
560	0.49053	0.00012	1140	0.42691	0.00011	1695	0.08525	2.1E-05
			1160	0.42124	0.00011	1700	0.07959	2E-05

Diameter of the microelectrode: 5 μm

Flow velocity: 1.15 cm/sec

 $\text{Fe}(\text{CN})_6^{3-}$ conc.: 21 mM

Raw data of Fig. 8

Curve (a)		
Verical Position (μm)	limiting current ($\times 10^7 \text{ A}$)	k (m/s)
0	0.0008	9.62E-07
2	0.002	2.41E-06
4	0.009	1.08E-05
6	0.0164	1.97E-05
8	0.0192	2.31E-05
10	0.0218	2.62E-05
12	0.0228	2.74E-05
14	0.0232	2.79E-05
16	0.0232	2.79E-05
18	0.0246	2.96E-05
20	0.0248	2.98E-05
30	0.026	3.13E-05
70	0.0306	3.68E-05
100	0.0306	3.68E-05
200	0.0306	3.68E-05
900	0.031	3.73E-05
1000	0.0312	3.75E-05
1500	0.0314	3.78E-05
2000	0.0316	3.8E-05

Curve (b)		
Verical Position (μm)	limiting current ($\times 10^7 \text{ A}$)	k (m/s)
2	0.0016	1.92E-06
6	0.0098	1.18E-05
8	0.013	1.56E-05
10	0.0176	2.12E-05
12	0.0184	2.21E-05
14	0.0178	2.14E-05
16	0.0172	2.07E-05
18	0.017	2.04E-05
20	0.0166	2E-05
22	0.0164	1.97E-05
24	0.0162	1.95E-05
26	0.0158	1.9E-05
28	0.0156	1.88E-05
30	0.0154	1.85E-05
40	0.019	2.29E-05
50	0.0154	1.85E-05
60	0.012	1.44E-05
70	0.0108	1.3E-05
80	0.012	1.44E-05
90	0.01	1.2E-05
100	0.009	1.08E-05
110	0.0076	9.14E-06
120	0.0186	2.24E-05
130	0.0206	2.48E-05
140	0.0264	3.18E-05
150	0.0162	1.95E-05
160	0.0288	3.46E-05
170	0.029	3.49E-05
180	0.029	3.49E-05
190	0.0292	3.51E-05
200	0.0292	3.51E-05
300	0.0298	3.58E-05
400	0.0286	3.44E-05
500	0.0292	3.51E-05
600	0.0298	3.58E-05
700	0.0308	3.7E-05
800	0.0312	3.75E-05
900	0.0314	3.78E-05
1000	0.0316	3.8E-05

Diameter of the microelectrode: 7 μm

Flow velocity: 0.41 cm/sec

 $\text{Fe}(\text{CN})_6^{3-}$ conc.: 22.4 mM

Raw data of Fig. 9

Verical Position (μm)	Curve (a)		Curve (b)	
	limiting current ($\times 10^7 \text{ A}$)	k (m/s)	limiting current ($\times 10^7 \text{ A}$)	k (m/s)
1000	0.346	0.000184	0.345	0.000184
900	0.344	0.000183	0.34	0.000181
800	0.328	0.000175	0.33	0.000176
700	0.312	0.000166	0.319	0.00017
600	0.286	0.000152	0.302	0.000161
500	0.235	0.000125	0.282	0.00015
450	0.221	0.000118	0.183	9.74E-05
400	0.183	9.74E-05	0.146	7.77E-05
350	0.168	8.94E-05	0.129	6.87E-05
300	0.152	8.09E-05	0.114	6.07E-05
280	0.143	7.61E-05	0.105	5.59E-05
260	0.135	7.19E-05	0.095	5.06E-05
240	0.127	6.76E-05	0.095	5.06E-05
220	0.12	6.39E-05	0.09	4.79E-05
200	0.113	6.01E-05	0.086	4.58E-05
180	0.106	5.64E-05	0.082	4.36E-05
160	0.098	5.22E-05	0.077	4.1E-05
140	0.088	4.68E-05	0.073	3.89E-05
120	0.078	4.15E-05	0.071	3.78E-05
100	0.066	3.51E-05	0.083	4.42E-05
80	0.052	2.77E-05	0.166	8.84E-05
60	0.047	2.5E-05	0.008	4.26E-06
40	0.034	1.81E-05	0.061	3.25E-05
20	0.025	1.33E-05	0.01	5.32E-06
0	0.008	4.26E-06	0.007	3.73E-06

Diameter of the microelectrode: 10 μm

Flow velocity: 1.58 cm/sec

 $\text{Fe}(\text{CN})_6^{3-}$ conc.: 24.8 mM

Raw data of Fig. 10

Diameter of the microelectrode: 10 μm

Flow velocity: 1.58 cm/sec

$\text{Fe}(\text{CN})_6^{3-}$ conc.: 24.8 mM

(a) $z=1000 \mu\text{m}$

k(m/s)	y (μm)											
x (μm)	0	25	50	75	100	125	150	175	200	225	250	
0	1.4E-04	1.4E-04	1.4E-04	1.4E-04	1.4E-04	1.4E-04	1.4E-04	1.4E-04	1.4E-04	1.4E-04	1.4E-04	1.4E-04
25	1.4E-04	1.4E-04	1.4E-04	1.4E-04	1.4E-04	1.4E-04	1.4E-04	1.4E-04	1.4E-04	1.4E-04	1.4E-04	1.4E-04
50	1.4E-04	1.4E-04	1.4E-04	1.4E-04	1.4E-04	1.4E-04	1.4E-04	1.4E-04	1.4E-04	1.4E-04	1.4E-04	1.4E-04
75	1.4E-04	1.4E-04	1.4E-04	1.4E-04	1.4E-04	1.4E-04	1.4E-04	1.4E-04	1.4E-04	1.4E-04	1.4E-04	1.4E-04
100	1.4E-04	1.4E-04	1.4E-04	1.4E-04	1.4E-04	1.4E-04	1.4E-04	1.4E-04	1.4E-04	1.4E-04	1.4E-04	1.4E-04
125	1.4E-04	1.4E-04	1.4E-04	1.4E-04	1.4E-04	1.4E-04	1.4E-04	1.4E-04	1.4E-04	1.4E-04	1.4E-04	1.4E-04
150	1.4E-04	1.4E-04	1.4E-04	1.4E-04	1.4E-04	1.4E-04	1.4E-04	1.4E-04	1.4E-04	1.4E-04	1.4E-04	1.4E-04
175	1.4E-04	1.4E-04	1.4E-04	1.4E-04	1.4E-04	1.4E-04	1.4E-04	1.4E-04	1.4E-04	1.4E-04	1.4E-04	1.4E-04
200	1.4E-04	1.4E-04	1.4E-04	1.4E-04	1.4E-04	1.4E-04	1.4E-04	1.4E-04	1.4E-04	1.4E-04	1.4E-04	1.4E-04
225	1.4E-04	1.4E-04	1.4E-04	1.4E-04	1.4E-04	1.4E-04	1.4E-04	1.4E-04	1.4E-04	1.4E-04	1.4E-04	1.4E-04
250	1.4E-04	1.4E-04	1.4E-04	1.4E-04	1.4E-04	1.4E-04	1.4E-04	1.4E-04	1.4E-04	1.4E-04	1.4E-04	1.4E-04

(b) $z=500 \mu\text{m}$

k(m/s)	y (μm)											
x (μm)	0	25	50	75	100	125	150	175	200	225	250	
0	1.3E-04	1.4E-04	1.5E-04	1.4E-04	1.4E-04	1.5E-04	1.5E-04	1.5E-04	1.5E-04	1.5E-04	1.4E-04	1.4E-04
25	1.3E-04	1.4E-04	1.4E-04	1.4E-04	1.5E-04	1.4E-04	1.5E-04	1.5E-04	1.5E-04	1.5E-04	1.5E-04	1.4E-04
50	1.4E-04	1.4E-04	1.4E-04	1.5E-04	1.5E-04	1.5E-04	1.5E-04	1.5E-04	1.5E-04	1.5E-04	1.4E-04	1.4E-04
75	1.4E-04	1.4E-04	1.5E-04	1.5E-04	1.5E-04	1.5E-04	1.4E-04	1.4E-04	1.4E-04	1.4E-04	1.4E-04	1.4E-04
100	1.4E-04	1.4E-04	1.5E-04	1.5E-04	1.4E-04	1.5E-04	1.4E-04	1.4E-04	1.5E-04	1.5E-04	1.4E-04	1.4E-04
125	1.4E-04	1.4E-04	1.4E-04	1.4E-04	1.5E-04	1.5E-04	1.5E-04	1.5E-04	1.5E-04	1.5E-04	1.5E-04	1.4E-04
150	1.4E-04	1.4E-04	1.5E-04	1.5E-04	1.5E-04	1.5E-04	1.5E-04	1.5E-04	1.5E-04	1.5E-04	1.4E-04	1.4E-04
175	1.4E-04	1.4E-04	1.5E-04	1.5E-04	1.5E-04	1.5E-04	1.4E-04	1.4E-04	1.5E-04	1.5E-04	1.4E-04	1.4E-04
200	1.4E-04	1.4E-04	1.4E-04	1.4E-04	1.5E-04	1.5E-04	1.5E-04	1.5E-04	1.5E-04	1.5E-04	1.4E-04	1.4E-04
225	1.4E-04	1.4E-04	1.5E-04	1.5E-04	1.5E-04	1.5E-04	1.5E-04	1.5E-04	1.4E-04	1.4E-04	1.4E-04	1.4E-04
250	1.4E-04	1.5E-04	1.5E-04	1.5E-04	1.5E-04	1.5E-04	1.4E-04	1.4E-04	1.5E-04	1.4E-04	1.4E-04	1.4E-04

Raw data of Fig. 10 (continued)

(c) $z=300\ \mu\text{m}$

k(m/s)	y (μm)										
x (μm)	0	25	50	75	100	125	150	175	200	225	250
0	8.2E-05	1.4E-04	1.7E-04	1.6E-04	1.7E-04	1.9E-04	1.9E-04	1.9E-04	2.0E-04	1.9E-04	2.0E-04
25	8.2E-05	1.6E-04	1.8E-04	1.9E-04	1.9E-04	1.9E-04	2.1E-04	2.1E-04	2.1E-04	2.0E-04	2.0E-04
50	1.7E-04	1.8E-04	1.9E-04	1.7E-04	1.9E-04	1.9E-04	1.9E-04	1.8E-04	2.0E-04	1.9E-04	1.7E-04
75	1.7E-04	1.9E-04	1.9E-04	1.7E-04	1.8E-04	1.9E-04	1.8E-04	1.7E-04	1.7E-04	1.9E-04	1.7E-04
100	1.8E-04	1.8E-04	1.8E-04	1.4E-04	1.7E-04	1.7E-04	1.7E-04	1.9E-04	1.7E-04	1.9E-04	1.8E-04
125	1.6E-04	1.8E-04	1.9E-04	1.7E-04	1.6E-04	1.8E-04	1.8E-04	1.8E-04	1.8E-04	2.0E-04	1.9E-04
150	1.6E-04	1.8E-04	1.8E-04	1.8E-04	1.5E-04	1.7E-04	1.9E-04	1.8E-04	1.8E-04	1.9E-04	1.9E-04
175	1.6E-04	1.8E-04	1.9E-04	1.9E-04	1.8E-04	1.7E-04	1.9E-04	1.8E-04	1.9E-04	1.8E-04	1.9E-04
200	1.7E-04	1.8E-04	1.9E-04	1.8E-04	1.8E-04	1.7E-04	1.7E-04	1.7E-04	1.9E-04	2.0E-04	1.9E-04
225	1.7E-04	1.8E-04	1.9E-04	1.7E-04	2.0E-04	2.0E-04	1.9E-04	1.9E-04	1.9E-04	8.3E-05	1.9E-04
250	1.6E-04	1.8E-04	1.8E-04	1.9E-04	1.9E-04	1.8E-04	1.9E-04	2.0E-04	1.2E-04	2.1E-04	1.9E-04

(d) $z=200\ \mu\text{m}$

k(m/s)	y (μm)										
x (μm)	0	25	50	75	100	125	150	175	200	225	250
0	3.2E-05	1.2E-04	1.2E-04	1.1E-04	1.4E-04	1.3E-04	1.5E-04	1.6E-04	1.4E-04	1.5E-04	1.7E-04
25	3.2E-05	1.3E-04	1.6E-04	1.7E-04	1.3E-04	1.5E-04	1.4E-04	1.6E-04	1.5E-04	1.7E-04	1.4E-04
50	1.5E-04	1.7E-04	1.7E-04	1.4E-04	1.7E-04	1.4E-04	1.6E-04	1.4E-04	1.4E-04	1.2E-04	1.4E-04
75	1.5E-04	1.6E-04	1.7E-04	1.2E-04	1.3E-04	1.3E-04	1.4E-04	1.2E-04	1.4E-04	1.3E-04	1.5E-04
100	1.5E-04	1.8E-04	1.3E-04	1.4E-04	1.0E-04	1.5E-04	1.5E-04	1.3E-04	1.5E-04	1.3E-04	1.7E-04
125	1.4E-04	1.4E-04	1.3E-04	1.5E-04	1.1E-04	1.5E-04	1.4E-04	1.6E-04	1.7E-04	1.6E-04	1.7E-04
150	1.4E-04	1.2E-04	1.6E-04	1.5E-04	1.1E-04	1.4E-04	1.3E-04	1.7E-04	1.6E-04	1.7E-04	1.4E-04
175	1.5E-04	1.4E-04	1.6E-04	1.4E-04	1.4E-04	1.4E-04	1.5E-04	1.5E-04	1.5E-04	1.5E-04	1.3E-04
200	1.3E-04	1.5E-04	1.6E-04	1.3E-04	1.4E-04	1.3E-04	2.6E-05	1.5E-04	1.5E-04	1.6E-04	1.3E-04
225	1.3E-04	1.6E-04	1.4E-04	1.5E-04	1.3E-04	2.8E-05	1.5E-04	1.5E-04	1.6E-04	1.4E-04	1.5E-04
250	1.2E-04	1.5E-04	1.3E-04	1.3E-04	6.3E-05	1.3E-04	1.5E-04	1.2E-04	1.7E-04	1.4E-04	1.5E-04

Raw data of Fig. 10 (continued)

(e) $z=100\ \mu\text{m}$

k(m/s)	y (μm)											
x (μm)	0	25	50	75	100	125	150	175	200	225	250	
0	1.5E-05	7.9E-05	1.1E-04	1.0E-04	1.2E-04	1.1E-04	1.4E-04	1.1E-04	1.4E-04	1.4E-04	1.4E-04	
25	1.6E-05	9.9E-05	1.3E-04	1.2E-04	1.3E-04	1.1E-04	1.5E-04	1.3E-04	1.7E-04	1.4E-04	1.6E-04	
50	1.5E-04	1.5E-04	1.6E-04	1.6E-04	1.3E-04	1.1E-04	1.3E-04	1.3E-04	8.6E-05	1.3E-04	1.4E-04	
75	1.4E-04	1.5E-04	1.4E-04	1.5E-04	1.1E-04	1.3E-04	1.2E-04	1.3E-04	1.4E-04	1.1E-04	9.1E-05	
100	1.3E-04	1.3E-04	1.2E-04	1.2E-04	1.0E-04	1.3E-04	1.5E-04	1.3E-04	1.3E-04	1.8E-04	1.1E-04	
125	9.4E-05	1.2E-04	1.1E-04	1.2E-04	1.2E-04	1.3E-04	1.3E-04	1.5E-04	1.3E-04	1.8E-04	1.5E-04	
150	9.3E-05	1.1E-04	1.1E-04	1.1E-04	1.1E-04	1.2E-04	1.2E-04	1.5E-04	1.5E-04	1.6E-04	1.7E-04	
175	1.0E-04	1.1E-04	1.4E-04	1.1E-04	1.4E-04	1.1E-04	1.3E-04	1.6E-04	1.5E-04	1.3E-04	1.6E-04	
200	1.1E-04	1.5E-04	1.5E-04	1.2E-04	1.3E-04	1.1E-04	1.4E-04	1.4E-04	1.6E-04	1.4E-04	1.5E-04	
225	6.4E-05	1.3E-04	1.3E-04	1.5E-04	1.5E-04	1.2E-04	1.7E-04	1.2E-04	1.3E-04	1.6E-04	1.2E-04	
250	9.1E-05	1.3E-04	1.1E-04	1.4E-04	1.1E-04	1.5E-04	1.3E-04	1.5E-04	1.2E-04	1.5E-04	1.3E-04	

(f) $z=50\ \mu\text{m}$

k(m/s)	y (μm)											
x (μm)	0	25	50	75	100	125	150	175	200	225	250	
0	2.5E-05	4.1E-05	1.2E-04	1.1E-04	5.9E-05	6.5E-05	9.3E-05	5.1E-05	1.3E-04	1.0E-04	1.5E-04	
25	2.5E-05	1.1E-04	8.7E-05	1.1E-04	1.0E-04	1.1E-04	1.3E-04	8.1E-05	8.5E-05	8.6E-05	8.0E-05	
50	8.8E-05	1.2E-04	6.1E-05	1.3E-04	1.4E-04	1.3E-04	9.9E-05	9.5E-05	8.5E-05	9.0E-05	7.6E-05	
75	7.6E-05	1.3E-04	6.3E-05	1.2E-04	1.2E-04	8.9E-05	1.2E-04	7.0E-05	8.2E-05	8.1E-05	7.4E-05	
100	7.3E-05	8.6E-05	1.3E-04	1.2E-04	1.1E-04	1.3E-04	1.1E-04	8.5E-05	9.5E-05	9.3E-05	7.2E-05	
125	6.7E-05	8.5E-05	1.5E-04	1.1E-04	1.1E-04	8.8E-05	1.2E-04	9.0E-05	8.8E-05	9.3E-05	1.2E-04	
150	1.1E-04	7.1E-05	1.0E-04	1.3E-04	1.2E-04	6.8E-05	8.2E-05	7.9E-05	1.5E-04	1.7E-04	1.6E-04	
175	6.9E-05	9.3E-05	1.3E-04	1.3E-04	7.3E-05	7.1E-05	7.8E-05	1.3E-04	1.4E-04	1.4E-04	1.2E-04	
200	8.5E-05	5.1E-05	1.1E-04	1.2E-04	8.9E-05	6.9E-05	7.7E-05	1.3E-04	1.4E-04	1.4E-04	1.4E-04	
225	8.9E-05	9.9E-05	1.1E-04	6.3E-05	8.7E-05	8.0E-05	8.5E-05	1.2E-04	1.3E-04	1.1E-04	1.2E-04	
250	7.1E-05	1.1E-04	8.5E-05	8.5E-05	6.4E-05	7.1E-05	7.2E-05	1.2E-04	1.3E-04	1.1E-04	1.9E-04	

Raw data of Fig. 11

Diameter of the microelectrode: 9 μm

$\text{Fe}(\text{CN})_6^{3-}$ conc.: 22.0 mM

(a) $V=1.40$ cm/sec

K (m/s)	y (μm)										
x (μm)	0	25	50	75	100	125	150	175	200	225	250
0	9.7E-05	9.8E-05	1.0E-04	9.8E-05	1.0E-04	1.0E-04	1.0E-04	9.8E-05	9.7E-05	1.0E-04	1.0E-04
25	9.7E-05	1.0E-04	1.0E-04	1.0E-04	1.0E-04	1.0E-04	1.0E-04	1.0E-04	1.0E-04	1.0E-04	1.0E-04
50	1.0E-04	1.0E-04	1.0E-04	1.0E-04	1.0E-04	1.0E-04	1.0E-04	1.0E-04	1.0E-04	1.0E-04	1.0E-04
75	1.0E-04	1.0E-04	1.0E-04	1.0E-04	1.0E-04	1.0E-04	1.0E-04	1.0E-04	1.0E-04	1.0E-04	1.0E-04
100	1.0E-04	1.0E-04	1.0E-04	1.0E-04	1.0E-04	1.0E-04	1.0E-04	1.0E-04	1.0E-04	1.0E-04	1.0E-04
125	1.0E-04	1.0E-04	1.0E-04	1.0E-04	1.0E-04	1.0E-04	1.0E-04	9.9E-05	1.0E-04	1.0E-04	1.0E-04
150	1.0E-04	1.0E-04	1.0E-04	1.0E-04	1.0E-04	1.0E-04	1.0E-04	9.5E-05	1.0E-04	1.0E-04	1.0E-04
175	1.0E-04	9.8E-05	1.0E-04	1.0E-04	1.0E-04	1.0E-04	1.0E-04	9.5E-05	1.0E-04	1.0E-04	1.0E-04
200	1.0E-04	1.0E-04	1.0E-04	1.0E-04	1.0E-04	1.0E-04	9.8E-05	1.0E-04	1.0E-04	1.0E-04	1.0E-04
225	1.0E-04	1.0E-04	1.0E-04	1.0E-04	1.0E-04	1.0E-04	1.0E-04	1.0E-04	1.0E-04	1.0E-04	1.0E-04
250	1.0E-04	1.0E-04	1.0E-04	1.0E-04	9.8E-05	1.0E-04	9.7E-05	9.8E-05	9.8E-05	1.0E-04	1.0E-04

(b) $V=0.88$ cm/sec

k(m/s)	y (μm)										
x (μm)	0	25	50	75	100	125	150	175	200	225	250
0	9.5E-05	9.0E-05	8.7E-05	8.5E-05	7.2E-05	8.9E-05	8.8E-05	9.0E-05	9.4E-05	8.1E-05	9.0E-05
25	9.5E-05	9.8E-05	1.0E-04	9.8E-05	9.8E-05	1.0E-04	9.8E-05	1.0E-04	1.0E-04	1.0E-04	1.0E-04
50	1.0E-04	1.0E-04	9.8E-05	9.8E-05	9.6E-05	9.8E-05	9.8E-05	9.8E-05	9.7E-05	1.0E-04	1.0E-04
75	9.8E-05	9.5E-05	9.0E-05	9.7E-05	9.7E-05	9.8E-05	9.5E-05	9.7E-05	9.8E-05	9.4E-05	9.8E-05
100	9.4E-05	9.4E-05	8.8E-05	9.5E-05	9.5E-05	9.8E-05	9.8E-05	9.8E-05	9.8E-05	9.7E-05	9.7E-05
125	9.3E-05	9.1E-05	8.7E-05	8.8E-05	9.4E-05	1.0E-04	1.0E-04	9.8E-05	9.8E-05	9.3E-05	9.4E-05
150	9.3E-05	8.0E-05	8.7E-05	8.8E-05	8.8E-05	9.3E-05	9.5E-05	9.3E-05	9.2E-05	9.5E-05	9.8E-05
175	9.0E-05	9.4E-05	9.0E-05	9.0E-05	8.5E-05	9.3E-05	9.2E-05	9.4E-05	9.5E-05	9.7E-05	9.7E-05
200	8.5E-05	9.1E-05	9.1E-05	9.1E-05	8.8E-05	8.7E-05	8.8E-05	8.8E-05	8.7E-05	8.8E-05	9.3E-05
225	8.6E-05	9.1E-05	8.8E-05	8.5E-05	8.6E-05	9.1E-05	8.9E-05	8.9E-05	9.4E-05	9.3E-05	9.0E-05
250	7.8E-05	8.5E-05	8.5E-05	5.4E-05	9.0E-05	9.1E-05	8.6E-05	9.0E-05	9.3E-05	9.1E-05	8.8E-05

Raw data of Fig. 11 (continued)

(c) $V=0.76$ cm/sec

k(m/s)	y (μ m)											
x (μ m)	0	25	50	75	100	125	150	175	200	225	250	
0	9.4E-05	8.2E-05	9.0E-05	8.5E-05	6.6E-05	8.2E-05	7.5E-05	9.0E-05	9.0E-05	8.4E-05	7.1E-05	
25	9.4E-05	9.7E-05	9.7E-05	9.7E-05	9.8E-05	9.7E-05	9.7E-05	9.7E-05	9.8E-05	9.8E-05	9.7E-05	
50	9.8E-05	9.8E-05	9.7E-05	9.7E-05	9.5E-05	9.5E-05	9.5E-05	9.7E-05	9.5E-05	9.8E-05	9.8E-05	
75	9.8E-05	9.4E-05	9.3E-05	9.6E-05	9.5E-05	8.8E-05	9.3E-05	9.4E-05	9.5E-05	8.8E-05	9.5E-05	
100	9.5E-05	9.5E-05	9.1E-05	9.4E-05	9.0E-05	8.8E-05	9.5E-05	9.8E-05	9.4E-05	9.5E-05	9.5E-05	
125	9.3E-05	9.1E-05	9.1E-05	9.4E-05	9.3E-05	8.8E-05	9.5E-05	9.7E-05	9.5E-05	8.5E-05	8.8E-05	
150	9.4E-05	9.2E-05	9.0E-05	9.4E-05	8.2E-05	7.9E-05	9.1E-05	9.0E-05	9.0E-05	9.4E-05	9.4E-05	
175	7.8E-05	9.3E-05	9.0E-05	8.8E-05	8.1E-05	8.8E-05	8.7E-05	8.8E-05	9.4E-05	9.3E-05	9.4E-05	
200	8.8E-05	8.8E-05	8.8E-05	8.4E-05	7.8E-05	8.3E-05	8.7E-05	7.8E-05	8.3E-05	8.0E-05	8.0E-05	
225	8.5E-05	8.8E-05	8.7E-05	9.1E-05	8.5E-05	8.5E-05	8.0E-05	8.2E-05	8.0E-05	8.7E-05	9.3E-05	
250	7.2E-05	8.3E-05	8.7E-05	8.4E-05	8.2E-05	8.5E-05	8.0E-05	8.1E-05	7.7E-05	7.4E-05	8.1E-05	

(d) $V=0.41$ cm/sec

k(m/s)	y (μ m)											
x (μ m)	0	25	50	75	100	125	150	175	200	225	250	
0	9.4E-05	7.4E-05	7.7E-05	7.4E-05	7.5E-05	8.2E-05	8.4E-05	8.2E-05	8.7E-05	6.7E-05	7.2E-05	
25	9.4E-05	9.8E-05	9.7E-05	9.8E-05	9.7E-05	9.7E-05	9.8E-05	9.8E-05	9.8E-05	9.8E-05	9.7E-05	
50	9.8E-05	9.8E-05	9.7E-05	9.7E-05	9.1E-05	9.3E-05	9.7E-05	9.1E-05	9.1E-05	9.7E-05	9.7E-05	
75	1.0E-04	9.4E-05	9.3E-05	9.4E-05	8.8E-05	9.4E-05	9.5E-05	8.9E-05	9.4E-05	9.4E-05	9.7E-05	
100	9.0E-05	9.4E-05	8.5E-05	9.3E-05	9.4E-05	9.0E-05	9.3E-05	9.5E-05	9.4E-05	9.4E-05	9.1E-05	
125	8.8E-05	8.8E-05	9.1E-05	8.5E-05	8.1E-05	8.1E-05	8.7E-05	9.3E-05	9.5E-05	9.0E-05	9.4E-05	
150	8.6E-05	9.1E-05	8.8E-05	8.2E-05	8.8E-05	8.2E-05	9.1E-05	9.0E-05	8.6E-05	9.4E-05	9.5E-05	
175	8.1E-05	8.8E-05	8.8E-05	8.5E-05	7.8E-05	8.4E-05	8.8E-05	8.8E-05	9.3E-05	9.0E-05	8.2E-05	
200	8.5E-05	8.5E-05	8.4E-05	8.2E-05	8.7E-05	8.2E-05	8.5E-05	8.7E-05	8.4E-05	8.4E-05	8.1E-05	
225	7.8E-05	8.5E-05	8.1E-05	8.7E-05	8.4E-05	8.6E-05	8.6E-05	9.0E-05	8.2E-05	7.5E-05	8.4E-05	
250	8.2E-05	7.8E-05	8.2E-05	8.2E-05	8.2E-05	8.3E-05	8.7E-05	8.6E-05	7.3E-05	9.0E-05	7.8E-05	

Raw data of Fig. 14

Vertical position (μm)	k (m/s)	Velocity (cm/s)	Vertical position (μm)	k (m/s)	Velocity (cm/s)	Vertical position (μm)	k (m/s)	Velocity (cm/s)
0	4.00E-05	0.012	670	1.91E-04	1.092	1340	2.00E-04	1.932
10	1.34E-04	0.032	680	1.91E-04	1.102	1350	2.00E-04	1.942
20	1.49E-04	0.052	690	1.91E-04	1.122	1360	1.99E-04	1.952
30	1.53E-04	0.062	700	1.91E-04	1.132	1370	2.00E-04	1.962
40	1.54E-04	0.082	710	1.91E-04	1.152	1380	1.99E-04	1.972
50	1.55E-04	0.092	720	1.92E-04	1.162	1390	2.00E-04	1.982
60	1.58E-04	0.112	730	1.91E-04	1.182	1400	1.99E-04	1.992
70	1.58E-04	0.132	740	1.91E-04	1.192	1410	2.01E-04	2.002
80	1.60E-04	0.142	750	1.93E-04	1.202	1420	2.01E-04	2.012
90	1.60E-04	0.162	760	1.93E-04	1.222	1430	2.00E-04	2.022
100	1.63E-04	0.182	770	1.92E-04	1.232	1440	2.01E-04	2.032
110	1.64E-04	0.192	780	1.93E-04	1.252	1450	2.01E-04	2.042
120	1.64E-04	0.212	790	1.92E-04	1.262	1460	2.01E-04	2.052
130	1.66E-04	0.232	800	1.93E-04	1.282	1470	2.01E-04	2.062
140	1.67E-04	0.242	810	1.94E-04	1.292	1480	2.02E-04	2.072
150	1.69E-04	0.262	820	1.94E-04	1.302	1490	2.00E-04	2.082
160	1.69E-04	0.282	830	1.95E-04	1.322	1500	2.01E-04	2.092
170	1.70E-04	0.292	840	1.95E-04	1.332	1510	2.00E-04	2.102
180	1.70E-04	0.312	850	1.94E-04	1.342	1520	2.00E-04	2.112
190	1.71E-04	0.332	860	1.94E-04	1.362	1530	1.99E-04	2.122
200	1.72E-04	0.342	870	1.95E-04	1.372	1540	2.01E-04	2.132
210	1.73E-04	0.362	880	1.95E-04	1.382	1550	2.00E-04	2.142
220	1.73E-04	0.382	890	1.93E-04	1.402	1560	2.01E-04	2.152
230	1.74E-04	0.392	900	1.94E-04	1.412	1570	2.01E-04	2.152
240	1.74E-04	0.412	910	1.94E-04	1.422	1580	2.01E-04	2.162
250	1.75E-04	0.432	920	1.93E-04	1.442	1590	2.02E-04	2.172
260	1.76E-04	0.442	930	1.93E-04	1.452	1600	2.01E-04	2.182
270	1.77E-04	0.462	940	1.93E-04	1.462	1610	2.02E-04	2.192
280	1.77E-04	0.482	950	1.94E-04	1.482	1620	2.02E-04	2.202
290	1.76E-04	0.492	960	1.95E-04	1.492	1630	2.02E-04	2.212
300	1.78E-04	0.512	970	1.94E-04	1.502	1640	2.02E-04	2.222
310	1.78E-04	0.532	980	1.95E-04	1.512	1650	2.02E-04	2.232
320	1.79E-04	0.542	990	1.95E-04	1.532	1660	2.02E-04	2.232
330	1.80E-04	0.562	1000	1.95E-04	1.542	1670	2.02E-04	2.242
340	1.80E-04	0.572	1010	1.95E-04	1.552	1680	2.01E-04	2.252
350	1.79E-04	0.592	1020	1.96E-04	1.572	1690	2.01E-04	2.262
360	1.81E-04	0.612	1030	1.97E-04	1.582	1700	2.02E-04	2.272
370	1.81E-04	0.622	1040	1.95E-04	1.592	1710	2.02E-04	2.282
380	1.81E-04	0.642	1050	1.96E-04	1.602	1720	2.02E-04	2.282
390	1.80E-04	0.662	1060	1.97E-04	1.612	1730	2.01E-04	2.292

Raw data of Fig. 14 (continued)

Vertical position (μm)	k (m/s)	Velocity (cm/s)	Vertical position (μm)	k (m/s)	Velocity (cm/s)	Vertical position (μm)	k (m/s)	Velocity (cm/s)
400	1.79E-04	0.672	1070	1.96E-04	1.632	1740	2.02E-04	2.302
410	1.83E-04	0.692	1080	1.96E-04	1.642	1750	2.02E-04	2.312
420	1.84E-04	0.702	1090	1.96E-04	1.652	1760	2.00E-04	2.322
430	1.84E-04	0.722	1100	1.97E-04	1.662	1770	2.00E-04	2.322
440	1.84E-04	0.742	1110	1.97E-04	1.682	1780	2.01E-04	2.332
450	1.85E-04	0.752	1120	1.98E-04	1.692	1790	2.01E-04	2.342
460	1.85E-04	0.772	1130	1.98E-04	1.702	1800	2.01E-04	2.352
470	1.84E-04	0.782	1140	1.97E-04	1.712	1810	2.02E-04	2.362
480	1.84E-04	0.802	1150	1.97E-04	1.722	1820	2.03E-04	2.362
490	1.86E-04	0.822	1160	1.98E-04	1.732	1830	2.03E-04	2.372
500	1.86E-04	0.832	1170	1.97E-04	1.752	1840	2.03E-04	2.382
510	1.86E-04	0.852	1180	1.99E-04	1.762	1850	2.03E-04	2.392
520	1.87E-04	0.862	1190	1.98E-04	1.772	1860	2.03E-04	2.392
530	1.87E-04	0.882	1200	1.98E-04	1.782	1870	2.03E-04	2.402
540	1.88E-04	0.892	1210	1.98E-04	1.792	1880	2.03E-04	2.412
550	1.88E-04	0.912	1220	1.99E-04	1.802	1890	2.00E-04	2.422
560	1.89E-04	0.922	1230	1.98E-04	1.812	1900	2.03E-04	2.422
570	1.89E-04	0.942	1240	1.98E-04	1.822	1910	2.04E-04	2.432
580	1.88E-04	0.952	1250	1.98E-04	1.842	1920	2.03E-04	2.442
590	1.89E-04	0.972	1260	1.98E-04	1.852	1930	2.03E-04	2.442
600	1.89E-04	0.982	1270	1.98E-04	1.862	1940	2.03E-04	2.452
610	1.91E-04	1.002	1280	1.99E-04	1.872	1950	2.04E-04	2.462
620	1.90E-04	1.012	1290	2.00E-04	1.882	1960	2.03E-04	2.472
630	1.89E-04	1.032	1300	1.99E-04	1.892	1970	2.03E-04	2.472
640	1.91E-04	1.042	1310	2.00E-04	1.902	1980	2.03E-04	2.482
650	1.91E-04	1.062	1320	1.99E-04	1.912	1990	2.04E-04	2.492
660	1.90E-04	1.072	1330	2.00E-04	1.922	2000	2.03E-04	2.492



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