Diffusion coefficients for Pseudomonas aeruginosa and Klebsiella pneumoniae
by Yeong-Chul Kim

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Chemical Engineering
Montana State University
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Abstract:
The effects of motility and aggregation on the diffusion coefficient for bacteria were studied in an aqueous system. The effects of capillary tube sizes, exposure times, and dilution rates on the diffusion coefficient were examined.

In general, motile cells can diffuse about 1000 times faster than non-motile cells. Pseudomonas aeruginosa, a motile cell, and Klebsiella pneumoniae, a non-motile cell, were used for this research. Diffusion coefficients were measured by the capillary tube assay developed by Adler (1969). From this procedure the diffusion coefficient of Pseudomonas aeruginosa was 2.1 x 10^-5 (standard deviation : 1.3 x 10^-5) cmys and that of Klebsiella pneumoniae was 0.9 x 10^-5 (standard deviation : 0.9 x 10^-5) cmVs. The diffusion coefficient of Pseudomonas aeruginosa was about 2.3 times higher than that of Klebsiella pneumoniae.

The Stokes-Einstein equation cannot be 'used for estimating the diffusion coefficients for Klebsiella pneumoniae and Pseudomonas aeruginosa. The experimental value for the diffusion coefficient of Klebsiella pneumoniae was about 2000 times higher than that (4.5x10^-9 cm^2 Vs) obtained from the Stokes-Einstein equation. This discrepancy was due to aggregation of Klebsiella pneumoniae or technical error from this experiment.

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AND *KLEBSIELLA PNEUMONIAE*

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ABSTRACT

The effects of motility and aggregation on the diffusion coefficient for bacteria were studied in an aqueous system. The effects of capillary tube sizes, exposure times, and dilution rates on the diffusion coefficient were examined.

In general, motile cells can diffuse about 1000 times faster than non-motile cells. *Pseudomonas aeruginosa*, a motile cell, and *Klebsiella pneumoniae*, a non-motile cell, were used for this research. Diffusion coefficients were measured by the capillary tube assay developed by Adler (1969). From this procedure the diffusion coefficient of *Pseudomonas aeruginosa* was 2.1×10^{-5} (standard deviation : 1.3×10^{-5}) cm^2/s and that of *Klebsiella pneumoniae* was 0.9×10^{-5} (standard deviation : 0.9×10^{-5}) cm^2/s. The diffusion coefficient of *Pseudomonas aeruginosa* was about 2.3 times higher than that of *Klebsiella pneumoniae*.

The Stokes-Einstein equation cannot be used for estimating the diffusion coefficients for *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. The experimental value for the diffusion coefficient of *Klebsiella pneumoniae* was about 2000 times higher than that (4.5×10^{-9} cm^2/s) obtained from the Stokes-Einstein equation. This discrepancy was due to aggregation of *Klebsiella pneumoniae* or technical error from this experiment.

CHAPTER 1

INTRODUCTION

Systems which are not in equilibrium tend toward equilibrium according to the second law of Thermodynamics. This phenomenon occurs due to a difference in the chemical potential of a component between one region in space and another. There are many factors that can give rise to a difference in chemical potential: concentration, temperature, pressure differences, and differences in potential caused by external sources (gravity, magnetic, etc.). The only driving force considered in this research is a difference in cell concentrations. Molar flux is proportional to the concentration gradient, and the proportionality factor is the diffusion coefficient.

It is very important to know the diffusion coefficient of cells, if one is to understand the initial events of microbial adhesion in an aquatic system. Microbial cells attach to substrata firmly and cause energy losses (i.e., heat, mass, and momentum transfer resistances), deterioration of water quality, and corrosion of metals. In other cases, attached cells can be used for biological wastewater treatment. Knowing the mechanism of cell attachment to surfaces is useful to the understanding of attached bioprocesses.

First of all we should know actual diffusion coefficients of cells in aquatic systems. Diffusion coefficients of cells available in the

literature are rare due to few experimental data, as only Escherichia coli, various Salmonella and a few other bacteria have been studied in recent years.

It is also important to know the difference in diffusion for motile and non-motile cells. Here we define the difference between motility and mobility. Colloidal particles, which are mobile, show Brownian motion. Dead cells or non-motile cells exhibit this motion. Living cells are said to be motile when they are able to perform autonomous displacement. Motile bacteria can generally move much faster than non-motile bacteria due to the movement of flagella. Motile bacteria can be attracted by some chemicals, termed "attractants" and repelled by some other chemicals, termed "repellents". These chemicals are detected by "chemoreceptors". This is chemotaxis. The chemical gradient affects the receptors and changes directions of the flagella. In this research, chemotaxis is not considered.

Goal and Objectives of Research

The goal of this research is to determine the diffusion coefficients for Pseudomonas aeruginosa, which is motile, and Klebsiella pneumoniae, which is non-motile.

Two objectives were formulated:

1) Establish a technique for determining bacterial diffusivities.
2) Investigate the effect of cell aggregation on measured diffusivities.

CHAPTER 2

LITERATURE SURVEY

Previous Research

Observation of chemotaxis of motile cells by Pfeffer in 1884 became the basis of quantitative assessment of bacterial motility to many researchers (Adler, 1972). Ogiuti (1936) measured the time taken for *Escherichia coli* to move over a known distance. Shoesmith (1960) developed a simple, rapid, quantitative method to measure bacterial motility, which consisted of counting the number of organisms that pass across a small aperture in a given time. He found that the count was proportional to the suspended cell concentration and also to the average speed of the bacteria. Table 1 shows his results.

Table 1. Cell Counts

Species	Cell Conc. (#/ml)	Count(1)	Suspending Solution (#/min)
Pseudomonas viscosa	2.0×10^9	70	Phosphate Buffer (pH:7.4)
Bacillus brevis	0.8×10^9	32	Phosphate Buffer (pH:7.2)
Escherichia coli	0.9×10^9	12	Phosphate Buffer (pH:7.2)

Note : (1) Count means the number of cells passing across a small aperture per minute

Vaituzis and Doetsch (1969) developed a motility track technique which is of value in studying the changes of direction, reversals, and rotations observed in motile behavior. In this technique a drop of

culture was placed on a glass slide, where he recorded bacterial movements for several cells in time and space on a single photograph. A mean velocity was calculated from the average of the five longest tracks found in one photograph. It was assumed that the track was made by an organism moving parallel to the cover slip and at a uniform velocity. Table 2 shows his result for the mean velocities of several organisms. Also, results of Adler and Dahl (1967) and Ogiuti (1936) are included in Table 2.

Table 2. Velocity of Various Bacteria

Organism	Mean Velocity (μm/s)	Flagella	Temp (^{0}C)
Pseudomonas aeruginosa	55.8	Monotrichous	30
Escherichia coli	16.5	Peritrichous	30
	25 (Ogiuti,1936)		
	28.8 (Adler & Dahl,1967)		35
Bacillus licheniformis	21.4	Peritrichous	30
Sarcina ureae	28.1	Peritrichous	30
Chromatium okenii	45.9	Lophotrichous	30
Thiospirillum jenense	86.5	Lophotrichous	30

Pseudomonas aeruginosa, Chromatium okenii, and Thiospirillum jenense, polar flagellated cells, appear to move more rapidly than cells with peritrichous forms (Escherichia coli, Bacillus licheniformis, and Sarcina ureae), although extensive and definitive studies have not been made.

Adler (1967,1973) developed a capillary tube assay to measure chemotaxis, based on Pfeffer's experiment. Adler and Dahl (1967) measured the motility of Escherichia coli in a capillary tube. They also measured the diffusion of glucose and verified the diffusion of glucose can be correctly described by the well-known diffusion equation.

Diffusion of glucose under conditions identical to those used for the study of bacterial motility was 4.722×10^{-6} - 6.111×10^{-6} cm^2/s. This shows a good agreement with 6.667×10^{-6} cm^2/s which Gladden and Dole (1953) have obtained at 25^0C. Segel et al. (1977) used capillary tube assay to get the average motility of bacterial populations, and he concluded that the assay could be operated with speed, simplicity, and sufficient accuracy and was a valuable tool in the assessment of motility. Slater and Powell (1981) used a video camera and recorder to monitor the passage of the cells through the capillary tube.

Nossal and Chen (1972) investigated cell movement by laser light intensity correlation spectroscopy. Nossal and Weiss (1973) used light scattering densitometry assay. Dahlquist, Lovely and Koshland (1972) developed a technique of obtaining detailed quantitative data on the chemotactic response in order to study its precise dynamics and to clarify the relationship to chemoreception in higher organisms. Cell concentration is determined by monitoring the intensity of light scattered by the bacteria, using a photomultiplier tube to measure the intensity of the laser light. Berg and Brown (1972) built a microscope which automatically followed individual cells to get tracking image. Stock (1978) used photon correlation spectroscopy.

Among the methods for determining diffusion coefficients for cells, methods which need laser light or video camera and recorder are difficult to use, and require special equipment. Therefore, the capillary tube assay was selected for investigating bacterial motilities in this research.

Model Equation

A classical diffusion equation based on a material balance for cells in three dimensions can be used to calculate diffusion coefficients for bacteria :

$$\frac{\partial C}{\partial t} = D_{AB} \left(\frac{\partial^2 C}{\partial X^2} + \frac{\partial^2 C}{\partial Y^2} + \frac{\partial^2 C}{\partial Z^2} \right) \qquad (1)$$

here C : Cell concentration

t : Time

X : Axis

Y : Axis

Z : Axis

D_{AB} : Diffusion coefficient

For one dimension, suitable for a long capillary tube with a small cross-sectional area, Eq. (1) reduces to the following :

$$\frac{\partial C}{\partial t} = D_{AB} \frac{\partial^2 C}{\partial X^2} \qquad (2)$$

The boundary and initial conditions of the diffusion equation are as follows :

Boundary condition (1) : $C = C_0$ @ $X = 0$ and $t \geq 0$

Boundary condition (2) : $C = 0$ @ $X = L$ and $t \geq 0$

Initial condition : $C = 0$ @ $t = 0$ and $0 \leq X \leq L$

The solution for Eq. (1) with the initial and boundary conditions is as follows (Segel, 1977) :

$$D_{AB} = \pi N^2 / (4 C_0^2 A^2 t) \quad (3)$$

where N : Total cell number in the capillary tube

C_0 : Suspended cell concentration

A : Cross-sectional area of the capillary tube

t : exposure time

Theoretical Equation

The Stokes-Einstein equation gives good estimates for the diffusion coefficients of large spherical molecules or particles in liquids (for example, polymer molecules or colloidal particles). Bacterial movement can be treated as diffusion of colloidal particles because colloidal particles range from approximately 10 A to 1 μm in diameter. Therefore the Stokes-Einstein equation can be used for estimating a diffusion coefficient for non-motile cells, but cannot be used for motile cells. The Stokes-Einstein equation is as follows :

$$D_{AB} = \frac{k_B T}{f} = \frac{k_B T}{6 \pi \mu_B R_A} \quad (4)$$

where k_B : Boltzman constant, 1.38062×10^{-23} J/K

f : Frictional coefficient

T : Temperature, K

μ_B : Viscosity of suspended medium

R_A : Radius of cell

Environmental Effects on Diffusion Coefficients for Cells

Variations in cultural conditions (i.e., temperature, pH, osmotic pressure, and nutritional level) may affect motility both quantitatively

and qualitatively. Adler and Templeton (1967) examined the effects of environmental conditions on the motility of Escherichia coli, and reported the following needs.

1) Chelating agents are necessary to maintain motility in the presence of traces of heavy metal ions
2) A buffer is necessary to maintain pH at the optimum for motility
3) An energy source is necessary to stimulate the motility

Chelating Agents

Motility is highly sensitive to inhibition by trace amounts of heavy metal ions. Amino acids are good chelating agents for metal ions. A chelating agent such as EDTA (ethylene-diamine tetraacetic acid) added with glucose and buffer allowed excellent motility. Bacteria which were highly motile completely lost their motility within 30 minutes when they were washed free from the medium and then placed into a medium which is lacking a chelating agent. Their motility was fully restored at once by adding EDTA or amino acid (Adler et al., 1967).

pH

Flagella are known to disintegrate into subunits at pH 3 to 4 (Adler and Templeton, 1967). Cells which were deliberately suspended in buffers with lower pH values had lower cell counts passing through small aperture (Shoesmith, 1960).

Energy Source and Oxygen

Motility requires oxygen if the particular substrate yields energy

only aerobically, but motility does not require oxygen if pathways are present for obtaining energy from a substrate anaerobically. Adler (1973) showed that *Escherichia coli* which grew without shaking were poorly motile and grew poorly, but longer shaking in the medium caused serious loss in viability (Adler and Dahl, 1967).

Temperature

An organism grown at above optimum temperature may be very weakly motile and have very few flagella because the synthesis of flagella may be prevented. But the growth of the organism may be almost normal. Ogiuti (1936) found that the optimum temperature decreased for the motility of Salmonella as the incubation time increased (Adler and Templeton, 1967). Transport itself is a process that is highly dependent upon temperature.

Viscosity

Shoesmith (1960) reported that a slight increase in viscosity of a suspending medium above that of a buffer solution had the effect of increasing bacterial velocity, whereas a further increase reduced it. Schneider and Doetsch (1974) showed bacterial motility for all motile flagellated bacteria increased in a more viscous solution, reached a maximum, and decreased with higher viscosities. Peritrichously flagellated bacteria had maximum velocity at higher viscosities than polar flagellated bacteria. Schneider and Doetsch (1974) indicated that it is a general behavioral phenomenon of motile bacteria.

Diffusivities Studied Previously

Table 3 shows diffusivities for organisms which several researchers have obtained under different environmental conditions. Table 4 shows the cell specificities to help qualify the results in Table 3.

Table 3. Diffusivities of Cells (cm^2/s)

Reference	Bacterial Species	Diffusivities (cm^2/sec)	Temp. (^0C)
Shoesmith (1960)			
	Pseudomonas viscosa	2.60×10^{-5}	23
	Bacillus brevis	3.39×10^{-5}	30
	Escherichia coli	3.77×10^{-6}	30
Adler and Dahl (1967)			
	Escherichia coli	7.0×10^{-5}	35
Nossal and Chen (1972)			
	Escherichia coli	5.0×10^{-9}*	25
Berg and Brown (1972)			
	Escherichia coli	8.7×10^{-7}	32
Thonemann and Evans (1976)			
	Escherichia coli	9.0×10^{-6}	35
Stock (1972)			
	Salmonella typhimurium	4.3×10^{-9}*	37
Nossal and Weiss (1973)			
	Salmonella typhimurium	4.4×10^{-5}	25
Lovely and Dahlquist (1975)			
	Salmonella typhimurium	1.3×10^{-5}	25
Segel *et al.* (1977)			
	Pseudomonas fluorescens	5.56×10^{-5}	?
Slater and Powell (1981)			
	Bacillus cereus	3.6×10^{-6} 4.6×10^{-9}*	37

(Note * : Diffusivities for non-motile cell)

Table 4. Cell Specificities

Bacterial Genus	Cell Size (µm)	Motility
Pseudomonas	0.5-1.0 by 1.5-4.0	Motile by polar flagella
Escherichia	1.1-1.5 by 2.0-6.0	Motile by peritrichous flagella or non-motile
Bacillus	0.3-2.2 by 1.2-7.0	Majority motile (Flagella typically lateral)
Salmonella		Motile by peritrichous flagella
Klebsiella	0.3-1.5 by 0.6-6.0	Non-motile

Shoesmith (1960) used nutrient agar for Pseudomonas viscosa and suspended the growth in phosphate buffer. He used peptone medium for Bacillus brevis and Escherichia coli, centrifuged, and suspended the deposits in phosphate buffer with NaCl. Also bacterial suspension were aerated by shaking. Diffusion coefficients for three cells were calculated by Eq. (3) under assumptions that cells diffused in only one direction such as in the case of capillary tube of 10 µm in diameter. Adler and Dahl (1967) grew Escherichia coli with shaking, and then centrifuged, washed, and resuspended the pellet in potassium phosphate buffer. They repeated this procedure three times. Potassium phosphate buffer, EDTA, $MgSO_4$, $(NH_4)_2SO_4$, and L-serine were filled in the capillary tube.

Nossal and Chen (1972) added 0.01 M $CuCl_2$ to a sample to cause cessation of the cell movement, therefore movement could be assumed to be that of large Brownian particles. Escherichia coli moved like non-motile cells, and a very low diffusion coefficient was obtained instead of a diffusion coefficient typical of motile cells.

Berg and Brown (1972) grew Escherichia coli at 35^0C with rotary shaking, washed the cells twice with sodium phosphate buffer solution,

and suspended the cells in a sodium phosphate buffer (pH 7.0). They used the basic equation to predict the diffusivity, which they obtained from molecular theory. They assumed that cell diameter was 2 μm and viscosity of suspension was 2.7 cp at 32^0C.

Thonemann and Evans (1976) developed their model equation using the experimental results of Adler and Dahl (1967) to get the diffusivity of cells. They tested three types of distribution (Maxwellian, Uniform, and Exponential) speeds, and suggested that

exponential distribution speeds was the best.

Stock (1978) grew cells at 37^0C with constant aeration, then centrifuged, washed, and resuspended in phosphate buffer or nutrient broth. He obtained non-motile bacteria by subjecting the bacteria to an acid wash at pH 1.5. He got very low diffusivity for Salmonella typhimurium, which is almost the same as that of a non-motile cell.

Environmental condition for Nossal and Weiss's research (1973) was the same as that of Dahlquist et al. (1972).

Slater and Powell (1981) aerated Bacillus cereus by agitation. Bacillus cereus are peritrichous, therefore its movement is slower than that of cells which is monotrichous or lophotrichous. The cells were not washed. Also the cells were treated with 0.1 M $CuCl_2$ for 2 hours to get non-motile cells.

CHAPTER 3

EXPERIMENTAL APPARATUS, METHODS, AND TECHNIQUES

Nutrient and Dilution Solutions

Table 5 shows the composition of the nutrient solution used in the *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* cultures. The composition of the dilution solution is the same as that of the nutrient solution except the dilution solution does not contain glucose.

Table 5. Nutrient Solution

Component	M.W.	Amount(mg/l)
Glucose	180.16	100.0
NH_4Cl	53.49	36.0
$MgSO_4 \cdot 7H_2O$	246.48	10.0
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	1235.86	0.005
$ZnSO_4 \cdot 7H_2O$	287.54	0.5
$MnSO_4 \cdot H_2O$	169.0	0.04
$CuSO_4 \cdot 5H_2O$	249.68	0.01
$Na_2B_4O_7 \cdot 10H_2O$	381.37	0.005
$FeSO_4 \cdot 7H_2O$	278.05	0.56
$(HOCOCH_2)_3N$	191.1	2.0
$CaCl_2 \cdot 2H_2O$	147.02	92.0
(or $CaCl_2$)	110.99	69.4
Na_2HPO_4	142.0	568.0
KH_2PO_4	136.09	544.0

Note : The pH of the solutions should be adjusted to 6.8 using strong acid or base.

Apparatus

Figure 1 shows the experimental system. Nutrient solution is used for growing cells, dilution solution for changing a cell concentration in the measurement chamber. The chemostat is used for obtaining constant effluent cell concentration. The measurement chamber is well mixed to meet a boundary condition (1), i.e., constant concentration at x=0.

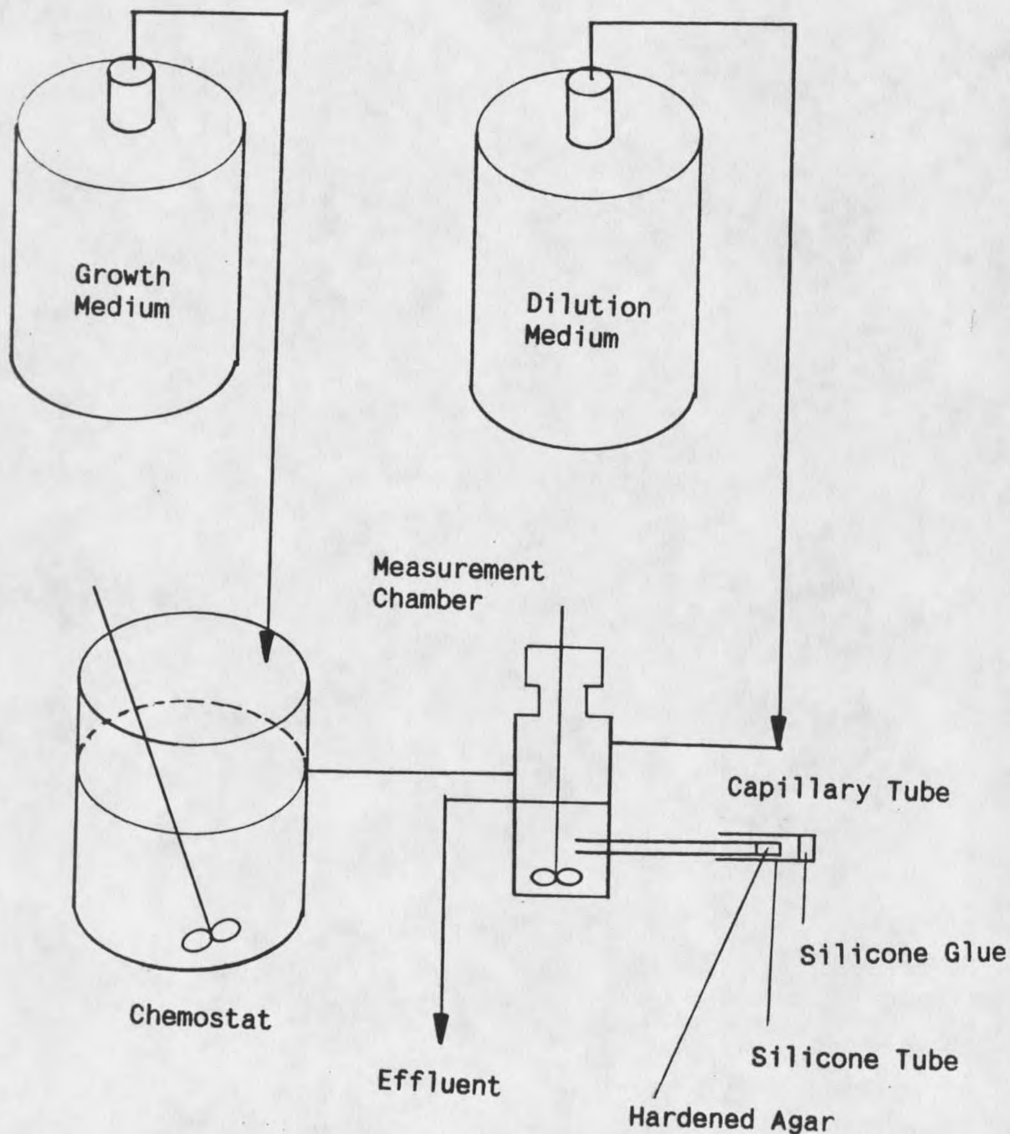

Figure 1. Apparatus

Experimental Procedure

1) Prepare the nutrient solution and dilution solution (10 liters of each) for Pseudomonas aeruginosa (Table 5). (Use a small spin bar (8 mm x 1.5 mm) to mix the contents in the measurement chamber)

2) Set up the chemostat, connect the chemostat inlet to nutrient solution, and the measurement chamber to chemostat effluent. Connect the dilution solution to the measurement chamber.

3) Make sure all connections are tight enough to be autoclaved.

4) Autoclave all experimental equipment for 1 hour at 121^0C and about 20 atmospheres.

5) After the experimental equipment cools down to room temperature, inoculate the pure culture of Pseudomonas aeruginosa to the chemostat. Before the culture is used, make sure that culture is pure. Verifications by API methods were shown in Appendix E.

6) Wait for 1 day, in order to grow the cells.

7) Turn on the pump for the nutrient solution and adjust the flow rate.

8) Wait 4 or 5 residence times to get a constant cell concentration from the chemostat effluent.

9) Turn on the pump and adjust the flow rate for the dilution solution.

10) Take 3-4 ml of the effluent from the measurement chamber.

11) Filter the effluent using membrane filter paper with pore size of 0.1 µm to filter out grown cells.

12) Inject this filtered effluent into a capillary tube (1.5-1.8 mm ID X 100 mm, Kimex no. 34502, Owens-Illinois, Toledo, Ohio) via a sterile syringe. Before being used, the capillary tube should be washed with

strong acid (Chromerge : Chromic-sulfuric acid cleaning solution, VWR)

13) Block the one end of capillary tubes with agar by plunging the tube into a Petri dish containing hardened agar.

14) Cover the end of the capillary tube blocked with agar with silicone tube (one-end sealed) to prevent the agar from moving out from the capillary tube by the head in the measurement chamber.

15) Plunge the open end of this capillary tube into the sample port of the measurement chamber (make sure there is no air bubbles in the capillary tube).

16) Remove the capillary tube from the measurement chamber after the designated time (10, 20, or 30 minutes).

17) Remove the entire contents of the capillary tube by applying air pressure on teh agar plug.

18) Stain the sample with acridine orange (Sigma) (or Hoechst 33258 or 33342 (Aldrich) for at least 1 hour) for 2 minutes.

19) Count the cell numbers by Image Analyzer (Quantimet 10, Cambridge / Olympus).

20) Repeat the same procedure from 12) with different sizes of capillary tubes (Size : 0.56 mm ID X 75 mm, Thomas Scientific, no. 2413K40 and Size :1.10 mm ID X 75 mm, Thomas Scientific, no. 2413K30).

21) Repeat the same procedure from 8) with different flow rates of nutrient and dilution solution.

22) Repeat the whole procedure with <u>Klebsiella</u> <u>pneumoniae</u>.

CHAPTER 4

RESULTS

Raw data from the counting procedure by the Image Analysis system are presented in Appendix A, for Pseudomonas aeruginosa, and Appendix B, for Klebsiella pneumoniae. Duplicate runs were always made for each designated time (10, 20, and 30 minutes). Ten fields per sample were taken to count the cell numbers by the Image Analysis system. Also average cell numbers per ml in a capillary tube were calculated based on a result in Appendices A and B. These calculated results are presented in Appendix C, for Pseudomonas aeruginosa, and Appendix D, for Klebsiella pneumoniae. Diffusion coefficients for P. aeruginosa and K. pneumoniae were calculated by Eq. (3) from data in Appendices C and D.
One sample calculation for Pseudomonas aeruginosa is presented here. Data for 10 minutes of exposure time (sample no. 1) in Appendix A and C were used.

$$D_{AB} = \frac{\pi N^2}{4\ C_0\ A^2\ t}$$

$$= \frac{\pi\ (2.53 \times 10^5)^2}{(4)\ (1.91 \times 10^8\ \#/ml)^2\ (0.0132\ cm^2)^2\ (10\ min)\ (60\ s)}$$

$$= 1.32 \times 10^{-5}\ cm^2/s$$

Diffusion Coefficients for Pseudomonas Aeruginosa

Table 6 shows diffusion coefficients calculated by Eq. (3) for five different suspended cell concentrations from data (1)-(5) in Appendix C. Flow rates of dilution solution were adjusted to change the cell concentration in the measurement chamber. The size of capillary tube and dilution rate were fixed during this experiment. Dilution rate was 0.19/h and the size of the capillary tube was 1.5 - 1.8 mm ID x 100 mm.

Table 6. Diffusivity with different cell concentrations (unit : 1×10^{-5} cm^2/s)

Cell conc. (#/ml)	Sample No.	Exposure Times			
		10 min.	20 min.	30 min.	Avg.
1.91×10^8	1	1.32	2.01	2.71	
	2	1.98	1.65	2.18	
	Avg.	1.65	1.83	2.45	1.975
4.95×10^7	1	0.975	0.967	1.05	
	2	0.730	1.14	2.03	
	Avg.	0.853	1.05	1.54	1.149
2.84×10^7	1	0.516	1.73	0.342	
	2	0.616	1.32	2.97	
	Avg.	0.566	1.53	1.66	1.249
2.00×10^7	1	-	1.13	4.36	
	2	-	11.6	2.55	
	Avg.	-	6.37	3.46	4.91
1.54×10^7	1	-	0.672	2.21	
	2	-	2.73	3.07	
	Avg.	-	1.70	2.64	2.171
SD		0.502	3.383	1.140	
Average		1.023	2.693	2.274	2.098

Note Avg. : Average
SD : Standard deviation
\- : Samples contaminated