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DIFFUSIVITY OF BACTERIA

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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 cell concentrations, capillary tube sizes, 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: 1.0×10^{-5}) cm^2/s and that of *Klebsiella pneumoniae* was 0.9×10^{-5} (standard deviation: 0.5×10^{-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 could not 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 the aggregation of *Klebsiella pneumoniae*.

Key words: *Diffusivity, Bacteria, Aggregation, Motile, Non Motile*

INTRODUCTION AND BACKGROUND

It is very important to know the diffusion coefficient of cells to understand the initial events of microbial adhesion in an aquatic system. Microbial cells attach to substrate 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 various bioprocesses.

1. 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, 1973]. 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. Vaituzis and Doetsch [1969] developed a motility track technique which is of value in studying the changes of direction, reversals, and rotations observed in cell motility. 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. From their work, *Pseudomonas aeruginosa, Chromatium okenii*, and *Thiospirillum jenense*, polar flagellated cells, appeared to move more rapidly than cells with peritrichous forms (*Escherichia coli, Bacillus licheniformis, and Sarcina ureae*), although extensive and definitive studies had 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. Dahlquist et al. [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. Nossal and Chen [1972] investigated cell movement by laser light intensity correlation spectroscopy. Nossal and Weiss [1973] used light scattering densitometry assay. 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. Stock [1978] used photon correlation spectroscopy. Slater et al. [1981] used a video camera and recorder to monitor the passage of the cells through the capillary tube.

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.

Diffusion coefficients of cells available in the literature are rare due to few experimental data, as only *Escherichia coli*, some *Salmonella* and a few other bacteria have been studied in recent years.

The goal of this research is to determine the diffusion coef-

ficients for *Pseudomonas aeruginosa*, which is motile, and *Klebsiella pneumoniae*, which is non-motile and investigate the effect of cell aggregation on measured diffusivities.

2. Theoretical Equation

The Stokes-Einstein equation gives good estimate for the diffusion coefficients of large spherical molecules or particles in liquids. Movement of non-motile bacteria can be treated as diffusion of colloidal particles because colloidal particles range from approximately 10 Å to 1 μm in diameter. Therefore, the Stokes-Einstein equation has been used for estimating a diffusion coefficient for non-motile cells.

The Stokes-Einstein equation is as follow:

$$D_{AB} = \frac{k_B T}{f} = \frac{k_B T}{6\pi\mu_B R_A} \tag{1}$$

where k_B is the Boltzman constant, f the frictional coefficient, T the temperature, μ_B the viscosity of suspended medium, and R_A the radius of cell.

3. Model Equation

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 forces, etc.). The only driving force considered in this research is a difference in cell concentrations.

A classical diffusion equation based on a material balance for cells in rectangular coordinates can be used to calculate diffusion coefficients [Fahien, 1983]:

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

where C is the cell concentration, t the exposure time, D_{AB} the diffusion coefficient of cells, and X, Y, Z the axes in rectangular coordinates.

For one dimension, suitable for a long capillary tube with a small cross-sectional area, Eq. (2) reduces to the following:

$$\frac{\partial C}{\partial t} = D_{AB}\frac{\partial^2 C}{\partial X^2} \tag{3}$$

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

Boundary condition (1) : $C = C_o$	at $X = 0$ and $t \geq 0$
Boundary condition (2) : $C = 0$	at $X = L$ and $t \geq 0$
Initial condition : $C = 0$	at $t = 0$ and $0 \leq X \leq L$

where C_o is the cell concentration in the measurement chamber, X the distance from the tip of the capillary tube, and L the length of the capillary tube.

The solution for Eq. (3) with the above initial and boundary conditions is as follow [Segel, 1977]:

$$D_{AB} = \pi N^2/(4C_o^2 A^2 t) \tag{4}$$

where N is the total cell number, and A the cross-sectional area of the capillary tube.

4. Environmental Effects on Diffusion Coefficients of Cells

Variations in cultural conditions (i.e., temperature, pH, osmotic pressure, and nutritional level) may affect cell 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 and Templeton, 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 an optimum temperature may be very weakly motile and have very few flagella because the synthesis of flagella may be prevented, although the growth of the organism may be almost normal. Ogiuti [1936] found that the optimum temperature for the motility of *Salmonella* decreased 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] also showed that 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.

4. Diffusivities Studied Previously

Table 1 shows diffusivities for organisms which several researchers have obtained under different environmental conditions. Table 2 shows the cell specificities to help qualify the results in Table 1.

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 suspensions were aerated by shaking. Diffusion coefficients for three cells were calculated by Eq. (4)

Table 1. Diffusivities of cells

Reference	Bacterial species	Diffusivities (cm^2/sec)	Temp. (°C)
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*	$5.0\times10^{-9*}$	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*	$4.3\times10^{-9*}$	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}	n.a.
Slater et al. [1981]	*Bacillus cereus*	3.6×10^{-6}, $4.6\times10^{-9*}$	37

Note : *denotes diffusivities for non-motile cell.
n.a. denotes 'not available'.

Table 2. 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*
Klebsiella	0.3-1.5 by 0.6-6.0	Non-motile

under assumptions that cells diffused in only one direction such as in the case of capillary tube. 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 and measured the diffusivity of cells 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°C 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°C. 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 speed was the best. Stock [1978] grew cells at 37°C 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 et al. [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.

EXPERIMENTALS

1. Nutrient and Dilution Solutions

Table 3 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 that the dilution solution does not contain glucose.

2. Apparatus

Fig. 1 shows the experimental system. Nutrient solution is used for growing cells, and dilution solution for changing a cell concentration in the measurement chamber. The chemostat is used for obtaining constant effluent cell concentration. The Adler's capillary tube assay was modified. The measurement chamber was installed just after the chemostat to get a constant cell concentration at the tip of the capillary tube. The measurement chamber should be well mixed.

3. Experimental Procedure

The nutrient solution and dilution solution were prepared and the chemostat was set up. All connections should be checked if those are tight enough to be autoclaved. All experimental equipment was autoclaved for 1 hour at 121°C and about 2 atmospheres. The pure culture of desired cells was inoculated to the chemostat. Verifications for pure culture were done by API methods in this work [Bergey, 1974]. Four or five residence times should be waited to get a constant cell concentration from the chemostat effluent.

The filtered effluent from chemostat was injected into a capillary tube (1.5-1.8 mm ID × 100 mm, Kimex no. 34502, Owens-Illinois, Toledo, Ohio) via a sterile syringe. It should be checked if there is no air bubbles in the capillary tube. Before being used, the capillary tube should be washed with strong acid (Chromerge : Chromic-sulfuric acid cleaning solution, VWR). The one end of capillary tubes should be blocked with agar by plunging the tube into a Petri dish containing hardened agar. The end of the capillary tube blocked with agar was covered with silicone tube (one-end sealed) to prevent the agar from

Table 3. 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
$(HOCOCH_2)_3$ N	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.

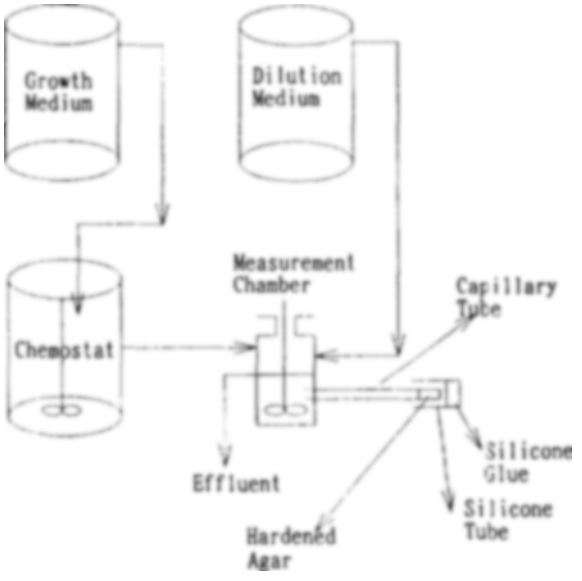

Fig. 1. Apparatus.

moving out from the capillary tube by the head in the measurement chamber. The open end of this capillary tube was plunged into the sample port of the measurement chamber. The capillary tube was removed from the measurement chamber after the designated time (10, 20, or 30 minutes). The entire content of the capillary tube was removed by applying air pressure on the agar plug. Ultrasonic cleaner was used to break the aggregates of cells. The sample was strained with acridine orange (Sigma) [or Hoechst 33258 or 33342 (Aldrich) for at least 1 hour] for 2 minutes. The cell numbers were counted by Image Analyzer (Quantimet 10, Cambridge/Olympus). The same procedure was repeated with different sizes of capillary tubes (Size : 0.56 mm ID × 75 mm, Thomas Scientific, no. 2413K40 and Size : 1.10 mm ID × 75 mm, Thomas Scientific, no. 2413K30). The same procedure was repeated for different flow rates of nutrient and dilution solution, meaning different dilution rates. The whole procedure was repeated with *Klebsiella pneumoniae*. All experiments were run at 28°C.

RESULTS

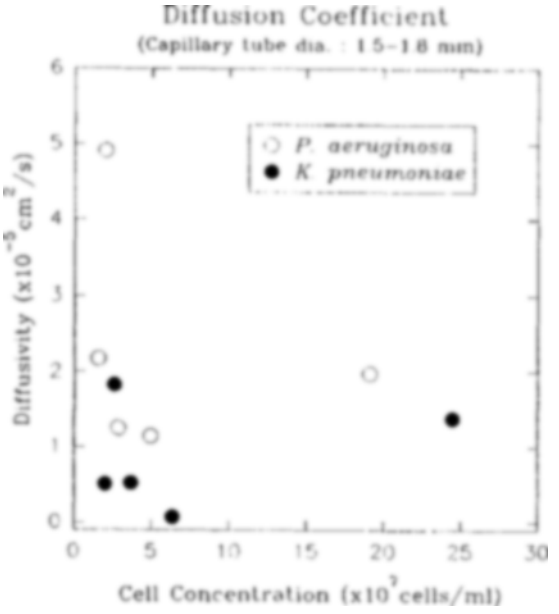

Fig. 2. Diffusivities of *P. aeruginosa* and *K. pneumoniae* with cell concentration.

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. With the averaged cell numbers, the diffusion coefficients for *P. aeruginosa* and *K. pneumoniae* were calculated by Eq. (4).

In Fig. 2 the average diffusion coefficient was plotted against suspended cell concentration for *P. aeruginosa* and *K. pneumoniae*. 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 × 100 mm. The slopes of *P. aeruginosa* and *K. pneumoniae* were found to be −0.044 and 0.023 by linear regression, respectively (unit : $\times 10^{12}$ cm^5/cell·s). The regression coefficient were 0.212 and 0.303, respectively. This was done to see the effect of cell concentration on its diffusion coefficient, meaning microbial interaction. Experimental results were too much deviated to find any trend.

In Fig. 3 a plot of the average diffusion coefficient versus diameter of tube can be found. Suspended cell concentrations (1.91×10^8 cells/ml for *P. aeruginosa* and 2.45×10^8 cells/ml for *K. pneumoniae*) in the measurement chamber were the same and the dilution rate (0.19 /h) was fixed throughout this experiment. The slopes for *P. aeruginosa* and *K. pneumoniae* were found to be −0.234 and −0.0635 by linear regression, respectively (unit : $\times 10^{12}$ cm^5/cell·s). The regression coefficients were 0.508 and 0.164, respectively. This experiment was performed to find whether capillary tubes used were appropriate for one-dimensional diffusion, i.e. for meeting Eq. (4).

In Fig. 4 a plot of the average diffusion coefficient versus dilution rate can be found. One size of capillary tube (1.5-1.8 mm I.D. × 100 mm) was used and suspended cell concentrations (1.91×10^8 cells/ml for *P. aeruginosa* and 2.45×10^8 cells/ml for *K. pneumoniae*) were fixed throughout this experiment. The slopes for *P. aeruginosa* and *K. pneumoniae* were found to be −8.56 and −3.684 by linear regression (unit : $\times 10^{12}$ cm^5/cell·

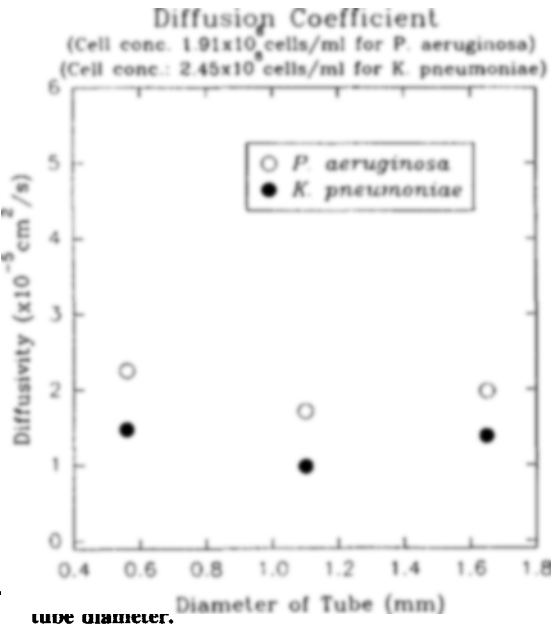

Fig. with tube diameter.

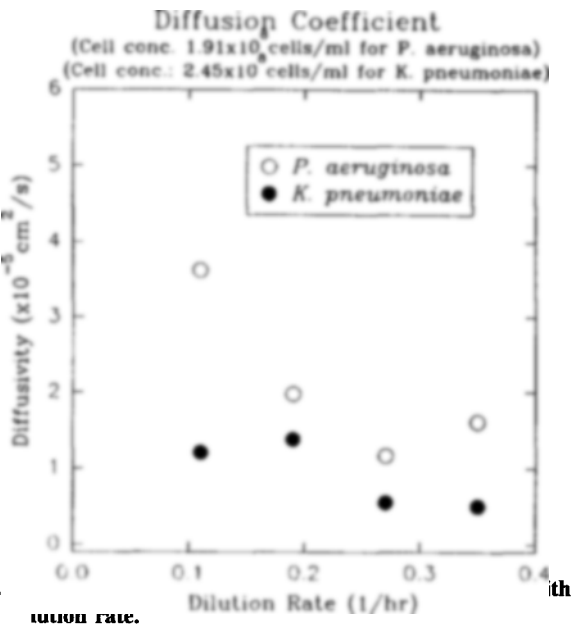

Fig. ith dilution rate.

Table 4. Size distribution and actual diffusivities of *Klebsiella pneumoniae*

Size of agg. (μm^2)	No. of agg.		$(D_{agg})_{cal}$ (cm^2/s)	Approx. cell no. in agg.	No. of agg. diffused in 20 min (cells/ml)		No. of cell diffused in 20 min (cells/ml)	
	Samp 1	Samp 2			Samp 1	Samp 2	Samp 1	Samp 2
0.11	0	7	14.7×10^{-9}	1	0	15094	0	15094
0.19	24	30	11.2×10^{-9}	1	13175	13175	13175	13175
0.34	48	64	8.49×10^{-9}	1	11471	11471	11471	11471
0.60	42	112	6.37×10^{-9}	2	9936	9936	19872	19872
1.08	54	113	4.75×10^{-9}	2	8580	8580	17160	17160
1.93	43	64	3.59×10^{-9}	4	7459	7459	29836	29836
3.44	13	266	2.67×10^{-9}	7	6433	6433	45031	45031
6.15	1	2	2.00×10^{-9}	13	5568	5568	72384	72384
10.99	1	2	1.50×10^{-9}	22	4822	4822	106804	106804
35.07	1	0	0.839×10^{-9}	71	3606	3606	256026	0
Total							5.71×10^{5}	3.30×10^{5}

Note: Agg. and samp. denote aggregate and sample.
$(D_{agg})_{cal}$ denotes diffusivity of aggregate calculated by Eq. (1), the Stokes-Einstein equation.

s). The regression coefficients were 0.824 and 0.846, respectively. Good correlation between the diffusion coefficient and dilution rate was obtained. It is believed that dilution rate affects the activity of cells.

DISCUSSION

All the diffusion coefficients for *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* obtained from this experiment were averaged and were 2.1×10^{-5} cm^2/s and 0.9×10^{-5} cm^2/s, respectively. The diffusion coefficient for *Pseudomonas aeruginosa* (2.1×10^{-5} cm^2/s) was consistent with those of other motile bacteria in Table 1, but the diffusion coefficient for *Klebsiella pneumoniae* (0.9×10^{-5} cm^2/s) was high, compared to the values calculated from Eq. (1), the Stokes-Einstein equation and compared to other non-motile cells. It is surmised that the difference is due to the aggregation of *Klebsiella pneumoniae*. The diffusion coefficient of spherical cells with a diameter of 1 μm in an aqueous medium, calculated by Eq. (1), the Stokes-Einstein equation (1cp and 28°C), was 4.5×10^{-9} cm^2/s.

Lots of aggregations for *Klebsiella pneumoniae* were observed during this experiment compared to those of *Pseudomonas aeruginosa*. Table 4 shows size distribution for *Klebsiella pneumoniae* under the conditions of this experiment. Two samples were taken from the measurement chamber after 20 minutes of exposure time. Size distributions for these two samples were obtained via the Image Analyzer. From the size distributions of *Klebsiella pneumoniae* the distribution of the diffusion coefficients were obtained. Data of the first two columns in Table 4 were obtained by the Image Analyzer. The data on the third column were calculated from Eq. (1), the Stokes-Einstein equation, using the values on column 1, which means the diffusion coefficient of cells with sizes shown on column 1. The values on column 4 were obtained based on the assumption that the cross-sectional area of one cell is 0.5 μm^2. The values on column 5 were calculated by Eq. (4) and those

on column 6 were obtained from the values on column 5 multiplied by those on column 4. With the total cell numbers diffused (column 6) the actual diffusion coefficient for *Klebsiella pneumoniae* was calculated by Eq. (4). $(D_{AB})_{actual}$'s for samples 1 and 2 were 2.10×10^{-5} and 7.03×10^{-6} cm^2/s, respectively. Therefore, the average actual diffusion coefficient calculated from the theoretical equation was 1.40×10^{-5} cm^2/s. This value shows better agreement with the experimental value (9.0×10^{-6} cm^2/s). It is because a large aggregate can diffuse much slower than a single cell, but the large aggregate contains a number of cells.

A long exposure time should not be used for this experiment because cells may grow in the capillary tube, and the movement of cells may be reduced under almost anaerobic conditions inside the capillary tube, and the cells can approach the opposite end of capillary tube, which means the boundary condition (2) cannot be applied to acquire the Eq. (4).

CONCLUSIONS

1. Actual diffusion coefficients for cells could be obtained from a modified capillary tube assay based on a method developed by Adler. From this research, the diffusion coefficients for *Pseudomonas aeruginosa*, a motile cell, and *Klebsiella pneumoniae*, a non-motile cell, are 2.1×10^{-5} and 0.9×10^{-5} cm^2/sec, respectively.

2. The Stokes-Einstein equation cannot be used for estimating actual diffusion coefficients for motile cells and non-motile cells that can form aggregates. From this research an actual diffusion coefficient for *Klebsiella pneumoniae* was 9.0×10^{-6} cm^2/s, which is almost in the range of diffusion coefficients for motile cells, but a diffusion coefficient calculated from the Stokes-Einstein equation was 4.5×10^{-9} cm^2/s, which is in a reasonable range of diffusion coefficients for non-motile cells (Table 1). The Stokes-Einstein equation may be used to estimate the diffusion coefficient of cells which are non-motile and do not form aggregates.

3. Diffusivities for *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* decrease with increasing dilution rates (Fig. 4) (The slopes for *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* were -8.56 and -3.684 (unit: 1×10^{12} $cm^2/cell\cdot s$). by linear regression), respectively.

4. The diffusion coefficients for *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* are almost independent of cell concentration and the capillary tube size.

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