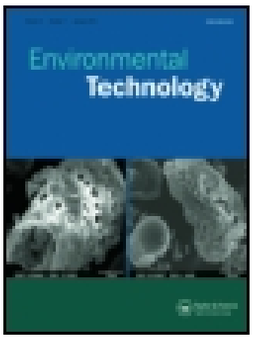

Environmental Technology

ISSN: 0959-3330 (Print) 1479-487X (Online) Journal homepage: www.tandfonline.com/journals/tent20

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To cite this article: T. Zhang & H.H.P. Fang (2005) Effective Diffusion Coefficients of Glucose in Artificial Biofilms, Environmental Technology, 26:2, 155-160, DOI: 10.1080/09593332608618574

To link to this article: https://doi.org/10.1080/09593332608618574

Published online: 11 May 2010.

Environmental Technology, Vol. 26. pp 155-160

EFFECTIVE DIFFUSION COEFFICIENTS OF GLUCOSE IN ARTIFICIAL BIOFILMS

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(Received 3 June 2004; Accepted 10 November 2004)

ABSTRACT

A technique using horizontal attenuated total reflection (HATR) by Fourier transform infrared (FTIR) spectrometry was demonstrated for the determination of effective diffusion coefficient (D_e) of an inert solute in biofilm. Glucose was the selected solute, and agarose (1%) hydrogel films containing various concentrations of activated sludge biomass were used to simulate the biofilm. The agarose films were formed on the surface of an internal reflection crystal in contact with a bulk solution containing 0.5M glucose. Glucose molecules diffused through the film by concentration gradient, and the glucose concentration at the biofilm-crystal interface was measured over time by HATR-FTIR. Based on the glucose concentration and film thickness, the D_e of glucose was calculated according to the Fick's Law. Results showed that D_e of glucose in the biomass-free agarose films averaged $6.46\pm0.21\times10^{-6}$ cm^2 s^{-1}, which is about 5% lower than the average reported D_e of glucose in water. The D_e of glucose decreased with increasing biomass concentration in the artificial biofilm. For the agarose films containing 0.45%, 0.90% and 1.80% of biomass, the D_e of glucose were lowered to $6.38\pm0.22\times10^{-6}$, $6.08\pm0.23\times10^{-6}$ and $5.62\pm0.17\times10^{-6}$ cm^2 s^{-1}, respectively.

Keywords: Agarose, biofilm, diffusion coefficient, glucose, FTIR

INTRODUCTION

Diffusions of substrate, nutrient, oxygen, metabolic products, etc., in the biofilm are of great interest to engineers. Such information is valuable for the modeling of biochemical reactions taking place inside the biofilm, as well as for the operation of bioreactors and the effective application of biocides.

Molecules diffuse in biofilm at slower rates than in the bulk solution, due to the presence of microbial cells, extracellular polymeric substances (EPS), metabolites, etc. [1]. The effective diffusion coefficient, D_e, of a chemical species may be determined from the concentration gradient in the biofilm under an unsteady state condition. Although several methods may be applied to the measurement of concentration gradient in biofilm, they all have intrinsic limitations. For example, confocal laser scanning microscopy (CLSM) is limited to species emitting fluorescence [1], nuclear magnetic resonance to species having ^{1}H, ^{13}C, ^{15}N, or ^{31}P as tracer [2], and microelectrodes to limited species such as H^+, S^{2-}, SO_4^{2-}, NO_3^-, Cl^- and dissolved oxygen. Thus, it is warranted to develop a method applicable to a broader variety of chemical species in biofilm.

In the medium infrared range, complex vibrational and rotational movements of molecules are excited by absorption of electromagnetic radiation. Those vibrations can be correlated with the single bonds or functional groups of a molecule, and thus are of great use in the identification of molecular species. Based on this principle, Fourier transform infrared (FTIR) spectrometry has been developed to become a common tool for the analysis of organic species. The absorption spectrum over the infrared range of interest is calculated from the interferogram by using the Fourier transform methods with elaborated mathematical algorithms. A technique using horizontal attenuated total reflection (HATR) by FTIR spectrometry has been developed and applied for the study of biofilm characteristics such as adsorption of polysaccharides [3], biofilm monitoring [4], microbial corrosion [5] and microbial identifications [6]. The same technique has also been applied for the study of diffusion of organic molecules in polymer films [7].

In this study, the authors wish to demonstrate that the HATR-FTIR technique may also be used to determine the effective diffusion coefficient of inert solutes in the biofilm.

THEORY

According to Fick's law, the molecular diffusion of a species in a biofilm under unsteady condition is expressed as follows:

$$\frac{\partial C_{(t,z)}}{\partial t} = De\frac{\partial^2 C_{(t,z)}}{\partial z^2} \quad \text{(i)}$$

where $C_{(t,z)}$ (concentration) is dependent upon t (time) and z (distance from the substratum-film interface), and D_e is the effective diffusion coefficient of the species. The diffusion described in Equation (i) is strictly due to concentration gradient of the species, which does not react with other chemical species in the biofilm.

For a film in contact with a bulk solution containing a non-interacting species at a concentration of C_b, the initial condition for Equation (i) is:

$$C_{(0,z)} = 0 \quad \text{(ii)}$$

Assuming the concentration at the film surface equals that of the bulk solution and a no-flux condition at the substratum interface, the boundary conditions for a film with a thickness of L are:

$$C_{(t,L)} = C_b, \quad \text{(iii)}$$

$$\frac{\partial C_{(t,0)}}{\partial z} = 0 \quad \text{(iv)}$$

The solution of Equation (i) with the aforementioned initial and boundary conditions may be expressed as follows [8]:

$$\frac{C_{(t,z)}}{C_b} = 1 - 2\sum_{n=0}^{\infty}\frac{(-1)^n}{(n+0.5)\pi}\exp[-(n+0.5)^2\pi^2\alpha]\cos[(n+0.5)\frac{\pi z}{L}] \quad \text{(v)}$$

in which C_b may be measured directly from the bulk solution. Equation (v) shows that the species concentration at any time and any depth depends on a single dimensionless parameter, α, given by

$$a = \frac{tD_e}{L^2} \quad \text{(vi)}$$

At the film-substratum interface, where $z = 0$, Equation (v) becomes

$$\frac{C_{(t,0)}}{C_b} = 1 - 2\sum_{n=0}^{\infty}\frac{(-1)^n}{(n+0.5)\pi}\exp[-(n+0.5)^2\pi^2\alpha] \quad \text{(vii)}$$

Since $C_{(t,0)}$, the species concentration at the film-substratum interface, may be measured by HATR-FTIR spectrometry, α may be determined from Equation (vii) by trial and error. Thus, based on Equation (vi) the effective diffusion coefficient, D_e, for a given film thickness L may be determined from the slope of α versus t.

MATERIALS AND METHODS

Measurement of $C_{(t,0)}$ by HATR-FTIR

The principle of HATR-FTIR measurement of $C_{(t,0)}$ is illustrated in Figure 1. A film is coated on an internal reflection element (IRE) that is made of a crystal of high refractive index. An IR beam enters from the IRE at an angle θ, reflects within the IRE and eventually exits from the other end. While traveling through the IRE, a certain fraction of the incident IR is absorbed by the chemical species in the biofilm located near the IRE interface.

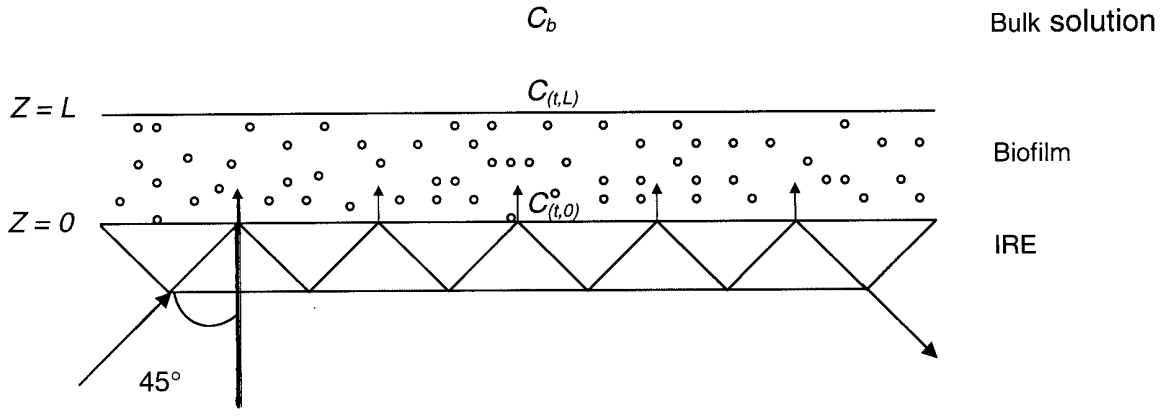

Figure 1. Horizontal attenuated of total reflection of an infrared ray in IRE.

The species concentration at the interface may be quantified from the IR absorption at certain wavenumber specific to that target species.

The depth of IR penetration into the film, d, is dependent upon the incident angle, θ, the wavelength (the reciprocal of wavenumber), λ, and the refractory index of the IRE, n, as shown in the following equation [6]:

$$d = \frac{\lambda}{\sqrt{2n\pi(\sin^2\theta - (1.33/n)^2}} \qquad \text{(viii)}$$

Concentrations of glucose in this study were analyzed using a FTIR spectrometer (Model Spectrum-One; Perkin Elmer, Foster City, CA) and the IRE was made of zinc selenide having a refractive index of 2.4. The incident angle was fixed at 45°, and the wavenumber ranged from 850 cm^{-1} to 3000 cm^{-1}. Based on Equation (viii), the depth of IR penetration into the film at the interface was 0.50-1.8 μm. All spectra were scanned with a resolution of 1 cm^{-1}.

Diffusion in Agarose Films

The diffusion experiments were conducted at 25±0.5°C. At first, the IR spectrum of glucose was scanned to find the wavenumber most sensitive for glucose. Glucose concentration was estimated from IR absorbance from the calibration curve established for that wavenumber.

Agarose (Pharmacia Biotech, Sweden) films were prepared by dissolving agarose in boiling water with gentle mixing for 10–15 min. The mixed solution was then decanted into a 30 ml cell (0.5×6×10cm), the bottom element of which was the zinc selenide crystal. The agarose film was allowed to solidify on the crystal surface for 60 minutes. A number of agarose solutions were dosed with given amounts of biomass and then used to form artificial biofilms for diffusion tests. Activated sludge obtained from a local wastewater treatment plant containing 80% volatile solids was concentrated by centrifugation before adding to the agarose solution. The artificial biofilms used in this study contained 1% agarose and activated sludge biomass at four concentrations: nil, 0.45%, 0.90% and 1.80%.

The approximate thickness of each artificial biofilm was controlled by the volume of agarose solution added. The exact thickness of each biofilm was however measured after the diffusion test. The biofilm was peeled off from the crystal, spread with yeast cells on both sides, and the vertical difference between the two layers of yeast cells was measured using a Zeiss microscope with computer-controlled z-stage with 0.1 μm accuracy.

For the diffusion tests, the 30 ml cell was filled with a 0.5 M glucose solution. The glucose concentration at the IRE interface increased with time due to molecular diffusion and was measured by HATR-FTIR. Three diffusion tests were conducted for biofilms containing a given concentration of biomass in order to check the reproducibility of the estimated D_e.

RESULTS AND DISCUSSIONS

In this study, the glucose concentration at the biofilm-IRE interface was measured based on the IR absorption at 1034 cm^{-1}. At that wavenumber not only was the IR absorption of glucose most intensive, but the interferences by other constituents of agarose and biomass were also minimal. For comparison, the IR absorption ranges were 1085-1114 cm^{-1} for polysaccharides, 1500-1745 cm^{-1} for proteins and 2850-3400 cm^{-1} for fatty acids [6].

Diffusion in Biomass-Free Agarose Gel Film

Diffusion of glucose in biomass-free agarose biofilms was conducted in three agarose films of 0.160, 0.222 and 0.241 cm respective thickness. Figure 2 illustrates the time profile of the glucose spectra at the biofilm-IRE interface. The glucose concentration at the interface was measured based on the absorption data at 1034 cm^{-1}. Figure 3a illustrates that α calculated from Equation (vii) increases linearly with time. From the slopes and the corresponding biofilm thicknesses, the D_e of glucose was estimated as $6.46\pm0.21\times10^{-6}$ cm^2 s^{-1} in the biomass-free films containing 1% agarose of various thicknesses. This value is comparable to the $6.58\pm0.18\times10^{-6}$ cm^2 s^{-1} reported for the D_e of glucose in 1% calcium alginate gel [9], but is 0.4-10.7% lower than the reported D_e values of glucose in water, $6.7\text{-}7.0\times10^{-6}$ cm^2 s^{-1} [10-12]. Similar degrees of reduction in diffusion coefficient were also reported in literature. Diffusion coefficient of fluorescein in an agarose gel [13] was 14% lower than that in water [1]. Diffusion coefficients of glucose, fructose, sucrose and lactose in 1% calcium alginate were 6%, 9%, 14% and 20% respectively, lower than the corresponding values in water [14].

Diffusion of Glucose in Artificial Biofilms

Figures 3b-3d illustrate that the diffusion parameter α increases linearly with time for diffusion of glucose in three artificial biofilms. The linearity as described by the Fick's Law suggests that there was little interactions between glucose and the bacterial cells. Based on film thickness and slopes in Figures 3b-3d, the D_e values of glucose in the artificial biofilm containing 0.45%, 0.90%, and 1.80% of activated sludge biomass were $6.38\pm0.22\times10^{-6}$, $6.08\pm0.23\times10^{-6}$, and $5.62\pm0.17\times10^{-6}$ cm^2 s^{-1}, respectively. These results show that diffusion coefficient of glucose was reduced in the presence of biomass in agarose films. Similar observations were reported for k-carrageenan gel [15]. The degree of reduction in diffusion coefficient increased with the biomass concentration in the biofilm. This is likely due to the obstruction effects of the bacterial cells on the diffusion of solute [16-18].

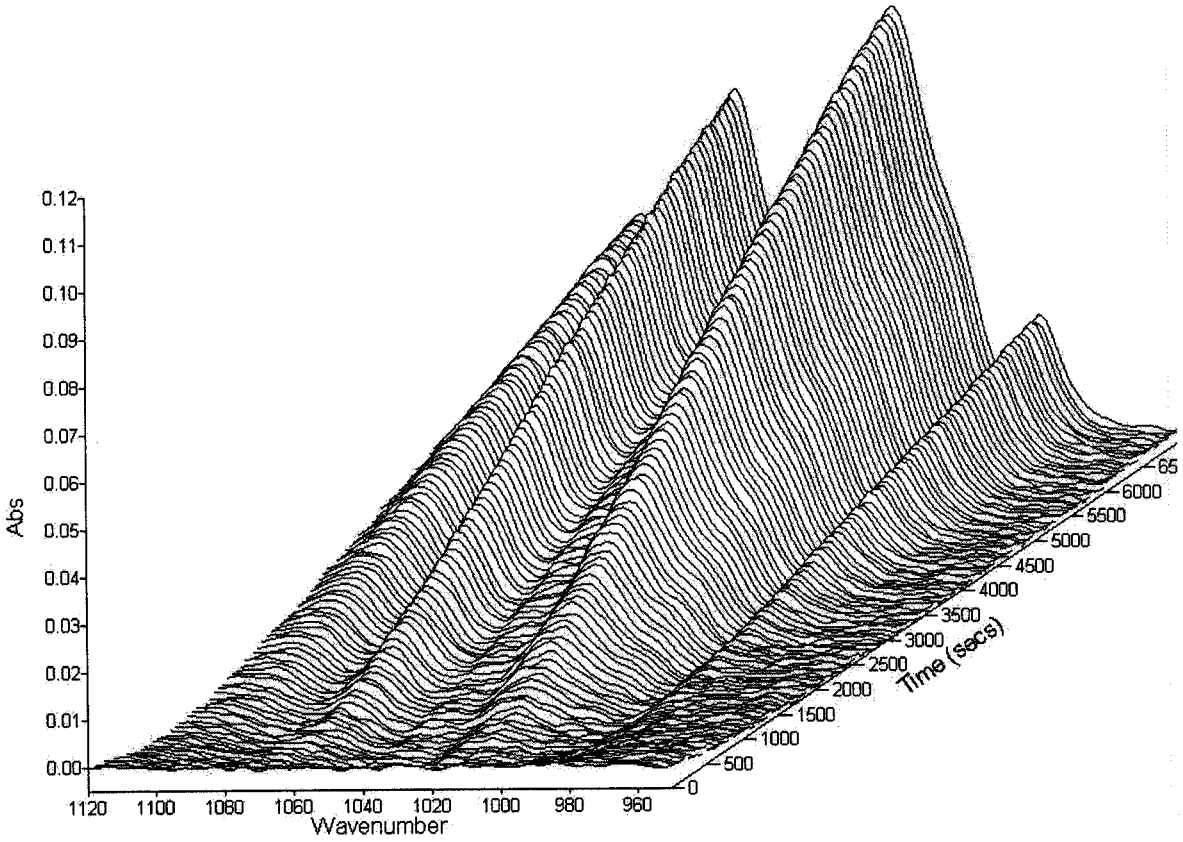

Figure 2. Time profiles of glucose spectra at the IRE-film interface.

Limitations and Potential of using HATR-FTIR

As demonstrated in this study, HATR-FTIR is a simple, effective and accurate means for the determination of solute diffusion coefficient in biofilm. The D_e value of glucose in three biomass-free agarose films with thickness varying from 0.160 to 0.241 cm had a standard deviation of 3%. However, this new method has three intrinsic limitations. Firstly, in order to follow the Fick's Law, the solute should have minimal interactions with other constituents in the biofilm, including bacterial cells and extracellular polymers. Secondly, the infrared absorption wavenumber of the target solute has to be specific, and the absorption is not interfered by the presence of bacterial cells and extracellular polymers. Such an absorption specificity for the target solute needs to be confirmed in order to obtain accurate estimation. Lastly, this new method requires the biofilm to be coated on the surface of a highly refractive crystal. As a consequence, a biofilm has to be removed from its substratum and put on the surface of the reflective crystal for the diffusion analysis, instead of being analyzed *in situ*.

On the other hand, although only the diffusion of a single solute was analyzed in this study, this new method is potentially applicable for the study of multi-species diffusion.

CONCLUSION

A novel technique using HATR-FTIR was demonstrated for the determination of effective diffusion coefficient of an inert solute in artificial biofilms. Results showed that the diffusion of solute in artificial biofilms of this study followed the Fick's Law. The D_e of glucose in the biomass-free 1% agarose films with thickness of 0.160-0.241 cm averaged $6.46\pm0.21\times10^{-6}$ cm^2 s^{-1}, which is about 5% lower than the average reported D_e of glucose in pure water. The D_e decreased with increasing biomass concentration in the artificial biofilm. For biofilms containing 0.45%, 0.90% and 1.80 % of biomass, the D_e of glucose were lowered, respectively, to $6.38\pm0.22\times10^{-6}$, $6.08\pm0.23\times10^{-6}$ and $5.62\pm0.17\times10^{-6}$ cm^2 s^{-1}.

ACKNOWLEDGEMENTS

The authors wish to thank the financial support given by Hong Kong Research Grants Council (HKU 7004/00E) and the Hong Kong University Development Fund for this study.

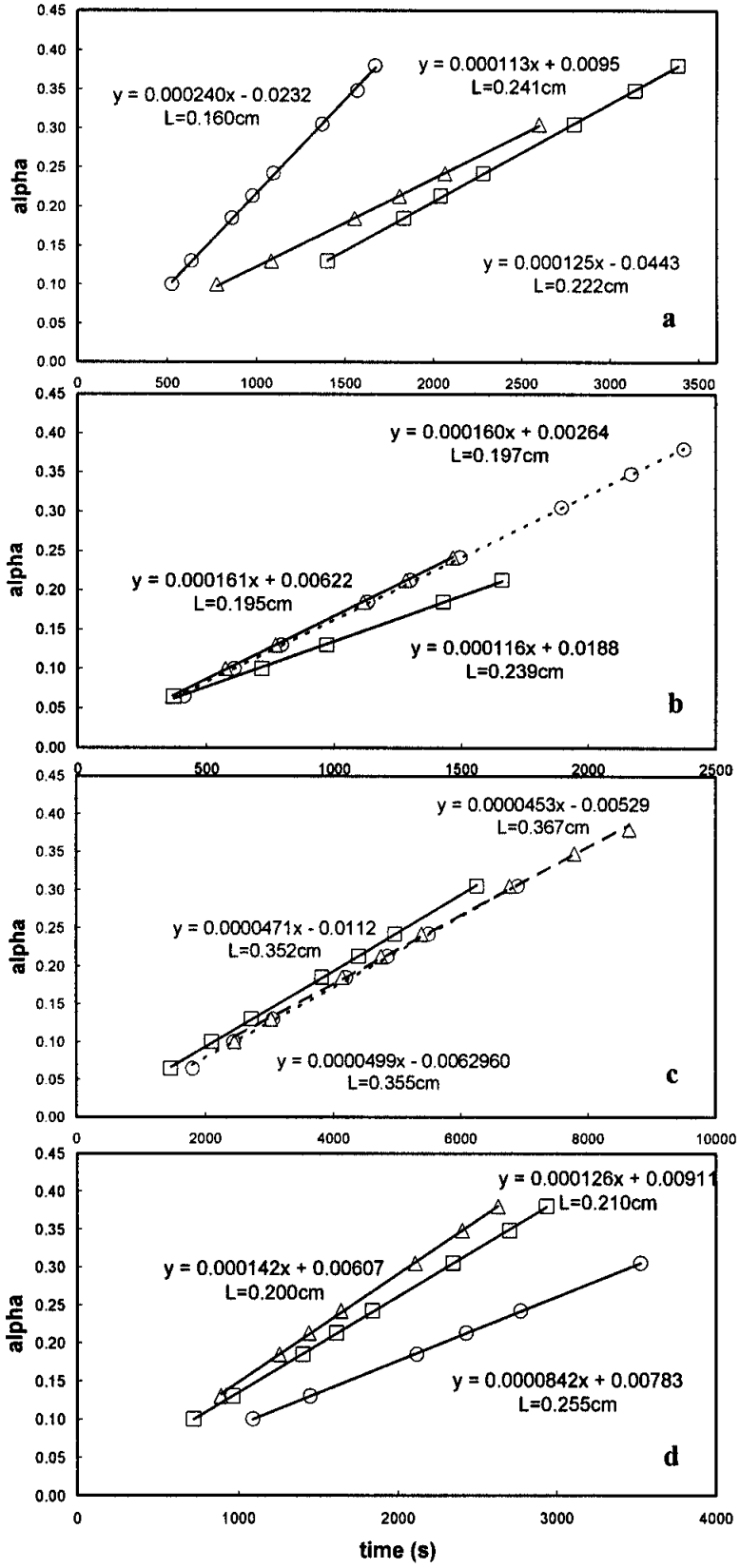

Figure 3. Plots of α versus time of glucose diffusion in artificial biofilms containing 1% agarose and various concentrations of activated sludge biomass: (a) nil, (b) 0.45%, (c) 0.90%, and (d) 1.80%.

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