Effect of biomass density on oxygen diffusivity measured inside biofilms with a MEA Sensor

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Abstract
The optimization of biofiltration technologies can be addressed improving the knowledge of the process taking place within biofilms, mainly biokinetics and mass transport. Biokinetics are usually defined using different methodologies, such as respirometric and titrimetric tests. Mass transport within biofilms is usually described as diffusion through a homogeneous phase, despite it is accepted that biofilms are very heterogeneous. Thus, a quantitative understanding of how biofilm structure is linked to mass transport is essential to develop reliable models. For this purpose different works have collected the results of various diffusion studies, proposing correlations between biofilm density and mass transport. However the reliability of these correlations, widely used in modeling works, is under suspect because data used in their construction are highly dependent on the experimental conditions where they were obtained. The goal of this paper was to experimentally quantify the effective diffusivity inside biofilms, using a specific microsensor, as function of biomass density, for a specific microbial population and substrate. In addition, biofilm diffusivity was measured at different hydrodynamic conditions. Combining both studies, an equation for the calculation of biofilm diffusivity, considering biomass density and liquid phase velocity, was proposed.

Keywords
Biofilm profiling, DO microsensor, mass transport modeling, biofilm diffusivity, diffusivity correlation.

INTRODUCTION

Biofilm performance is the key stage in the removal of pollutants in most of biofiltration technologies, both in liquid and gaseous wastes treatment. The knowledge of biodegradation mechanisms is required in the design of bioreactors and in the control of their operation. In this regard modeling has become a useful tool for the study and selection of the optimal operating conditions.

Modeling of biofilms is usually divided into two steps, mass transport of pollutants and substrates through the biofilm (Ning et al. 2012) and biochemical reaction (biokinetics) (Zhou et al. 2012). Since biofiltration optimization depends on models quality, much of the research efforts are focused on the enhancement of their reliability.

Biokinetics models have been extensively characterized for a wide range of pollutants by different studies, such as respirometric and titrimetric studies, performed using suspension cultures (Mora et al. 2014). Moreover, although diffusional resistance is neglected in these studies, it has been shown that the use of these biokinetic parameters is a suitable approximation in biofilms modeling. Hence, improvement of biofiltration models relies mainly in the increase of available mass transport information within biofilms. Mass transport through biofilms is commonly described using diffusional models, following Fick's laws, with diffusivity as mass transport coefficient (Fu et al. 1994). An efficient application of these models requires a quantitative understanding of how biofilms structure is linked to mass transport.

In this sense different works have focused their efforts on developing correlations for the calculation of diffusivity within biofilms considering biomass density (Fan et al. 1990; Hinson and Kocher 1996; Horn and Morgenroth 2006; Zhang and Bishop 1994a). However, these correlations presented some reliability problems. Correlations based on experimental results were developed from scarce data (Horn and Morgenroth 2006), and some of them used theoretical approaches (Hinson and Kocher 1996) or were established from theoretical studies (Zhang and Bishop 1994a), due to the difficulty of quantifying diffusivity through biofilms. The most used correlation was developed from the results of a literature review (Fan et al. 1990), using diffusivity data of various substrates in different biological systems, both in biofilms and biomass granules. Therefore these correlations can be only considered as an approximation.

The goal of this study is to experimentally quantify the diffusivity inside biofilms as a function of biomass density, using a single microbial population and substrate. By adjusting the hydrodynamic conditions of the reactor where biofilms were cultivated, a biomass density gradient along the biofilms was obtained. Furthermore, using a DO-MEA microsensor (Moya et al. 2014) specially designed for biofilm profiling, oxygen effective diffusivity was estimated from oxygenation profiles with a high spatial resolution (50 μm). Data obtained in these measurements were used in the development of an experimental correlation.

On the other hand, it is known that mass transport is also affected by the hydrodynamic conditions of the fluids involved in the system (liquid-biofilm). In this study, considering that biofilms are usually approximated as static systems, the evolution of diffusivity within biofilms as a function of liquid phase velocity over the biofilm was analyzed. These results were also used to further develop an empirical correlation as a function of biomass density and liquid phase velocity.

MATERIALS AND METHODS

DO-MEA microsensor

The DO-MEA microsensor (Moya et al. 2014), based on oxygen amperometric principle (Mottola 1978), was designed to obtaining dissolved oxygen (DO) concentration profiles of 1-mm depth via a single measurement. The microsensor was produced in the clean room facilities at Barcelona Microelectronics Institute (Spain), using photolithography techniques (Bonilla et al. 2011; Gabriel et al. 2007; Guimera et al. 2012). Sensor fabrication and performance description can be found in Moya et al. (2014).

Development of a heterotrophic biofilm

Diffusivity measurements from oxygenation profiles recorded by DO-MEA microsensor were conducted through an aerobic heterotrophic biofilm grown in a flat plate bioreactor (FPB). The FPB was manufactured in methacrylate (PMMA) as described in Lewandowski & Beyenal (2007). The reactor startup and operation are described in Guimerà et al. (2014).

Mass transport was studied as function of biofilms structures along the reactor. For this purpose a density profile, ranging from 10 to 60 $gVSS \cdot L^{-1}$, was obtained along the biofilm, by varying the environmental conditions such as substrate load and liquid velocity (Horn and Morgenroth 2006). In order to complete biofilm structure characterization, the biomass density was measured along the biofilm by protein analysis (Bradford 1976).

Measurement of biofilm diffusivity

Oxygen effective diffusivity within biofilms was determined experimentally using DO microsensors, by recording the oxygenation profiles and curve-fitting of experimental data with a mass transport model (Guimerà et al. 2014).

Dynamic oxygenation profiles

Dynamic oxygenation profiles, used in diffusivity determination, were obtained from a single DO-MEA measurement. These measurements were made as is described in Guimerà et al. (2014)

In order to obtain profiles where DO changes were only the result of mass transport, these profiles were conducted on deactivated biofilms, since diffusion through biofilms was not affected by deactivation (Matson and Characklis 1976). Bioactivity was prevented by recirculating a 300 $mg \cdot L^{-1}$ NaN_3 solution during 1h.

Biofilm modeling

A mass transport model within the biofilm was required to quantify oxygen diffusivity. It is known that biofilms are heterogeneous and complex systems (Zhang and Bishop 1994b), in which mass transport is strongly affected by biofilm structure (Bishop et al. 1995). However, the heterogeneous diffusion can be modelled as homogeneous by including the effect of biofilm structure into a unique parameter.

The mass transport was described using a non-steady state diffusion model (Fick's second law) (Eq. 1), with a diffusivity coefficient that is a function of biomass density.

$$\frac{dC}{dt} = D_B \cdot \frac{\partial^2 C}{\partial x^2} \qquad \text{Eq. 1}$$

Where C is the oxygen concentration in $mg \cdot L^{-1}$, t is time in seconds, D_B is oxygen diffusivity coefficient in biofilm in $cm^2 \cdot s^{-1}$ and x is the biofilm depth from liquid-biofilm interface in cm.

As biofilms are mainly composed by water, diffusivity inside biofilms is usually presented as relative diffusivity (Beyenal and Lewandowski 2000; Fu et al. 1994), relating solute diffusivity within biofilm with the solute molecular diffusivity in water (Eq. 2).

$$D_r = D_B \cdot D_W^{-1} \qquad \text{Eq. 2}$$

Where D_r is the relative diffusivity and D_W is the molecular diffusivity in water. D_r can be used for the calculation of different solutes diffusivities, using Eq. 2, if the size of the solute used in D_r determination, oxygen in this paper, is similar to the new solute (Stewart 1998). Considering this, D_r

can be defined as the dimensionless biofilm diffusivity, ranging between 1 (diffusivity in water) and 0 (no diffusion).

RESULTS

Oxygen diffusivity estimation within biofilm

Dynamic oxygenation profiles procedure was applied at different points along the biofilm grown in the FPB. Using the DO-MEA microsensor the reoxygenation of the biofilm at different depths inside a biofilm was recorded over time, obtaining an experimental oxygen distribution within the biofilm. The mass transport model (Eq. 1) was used in a nonlinear optimization technique, based on the Nelder-Mead method, to estimate the average oxygen diffusivity at the monitored biofilm section. In the experimental and optimized distributions, at a biomass density of 22 g VSS·L^{-1} and for a flow velocity of 9.88 m·h^{-1}, are shown.

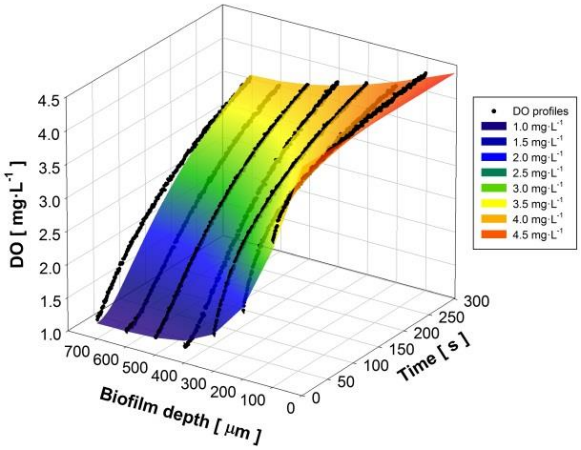

Figure 1. Simulated (mesh) and experimental (circle) oxygenation profiles obtained in a single measurement used in the determination of diffusivity in a biofilm section.

Figure 1 reveals the oxygen distribution through the biofilm, decreasing, at the beginning of the experiment, from 2.5 mg·L^{-1} on the biofilm surface to 1 mg·L^{-1} at a depth of 700 µm. Oxygenation curves at the different depths also showed a smooth slope of approximately 1.5·10^{-3} mg·L^{-1}·s^{-1}. At the end of the oxygenation, DO content ranged from 4.5 mg·L^{-1} at the liquid-biofilm interface to 4 mg·L^{-1} at the deeper zone. Figure 1 also showed a good fitting between the experimental DO distribution and the simulated one, resulting in a reliable estimation of oxygen diffusivity. Using the molecular oxygen diffusivity in water, (Nguyen et al. 2014), oxygen diffusivity can be converted into biofilm diffusivity.

Biofilm diffusivity at different biomass densities

Biofilm heterogeneity can be introduced into mass transport theory linking diffusion rate with biofilm structure, by relating the biofilm diffusivity with a macroscopic structural parameter, such as biomass density or porosity (Fan et al. 1990; Hinson and Kocher 1996; Horn and Morgenroth 2006; Zhang and Bishop 1994a).

In this study, biofilm diffusivity was quantified at different points of the biofilm, from the inlet to the outlet of the FPB. Mass transport results were correlated with the biomass density profile measured along the biofilm by protein analysis, as shown in Figure 2a.

Results presented in Figure 2a revealed a clear correlation between diffusivity and biomass density within biofilms. It was observed that biofilm diffusivity decreased, almost linearly, from 80 to 32% of the molecular diffusivity in water when biomass density increased from 9 to 33 g VSS·L^{-1}. In addition, results showed that for biomass densities over 50 g VSS·L^{-1}, the mass transport within biofilm was strongly limited, resulting in biofilm relative diffusivities below 5%. This trend can be explained because a higher biofilm density resulted in a decrease of biofilm porosity and thus less open volume was available to the substrate to diffuse through the biofilm (Fan et al. 1990; Zhang and Bishop 1994b).

The comparison of these experimental results with the available correlations for biofilm diffusivity estimation, shown in Figure 2b, is discussed in the Comparison to experimental correlations chapter.

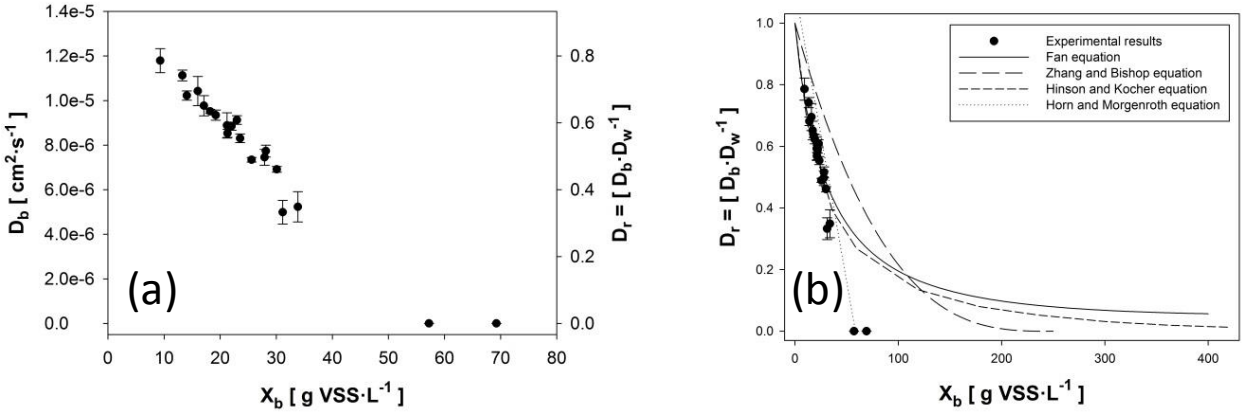

Figure 2. (a) Oxygen diffusivity (D_b) and biofilm diffusivity (D_r) in function of biofilm densities. The experiments were performed operating the reactor at a flow velocity of 9.88 m·h^{-1}. (b) Biofilm diffusivities measured at different biomass densities versus different available correlations for biofilm diffusivity estimation.

Biofilm diffusivity at different liquid velocities

Although mass transport within biofilms is mainly influenced by their structure, commonly compiled in a macroscopic parameter such as biofilm density, it is known that diffusion is also affected by other operational conditions. In this sense hydrodynamics has a clear impact on mass transport. Biofilm diffusivity was evaluated, with dynamic oxygenation procedures, at different reactor hydrodynamic conditions, by adjusting the liquid recirculation flow rate, and keeping constant the remaining conditions. In Figure 3 the biofilm diffusivity measured, in a biofilm section of 21.2 g VSS·L^{-1}, at different liquid phase velocities is shown.

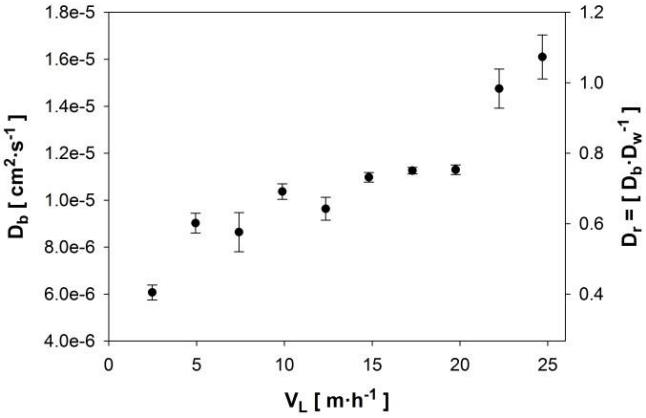

Figure 3. Results of the average effective diffusivity (●) in a biofilm density of 21.20 g VSS^{-1}·L^{-1}, calculated from oxygenation profiles, at different flow velocities

An important effect of hydrodynamics conditions on mass transport within biofilms can be observed in Figure 3. The results showed three different behaviors throughout the studied flow velocity range. At flow velocities below 10 m·h^{-1} relative diffusivity decreased from 0.7 to 0.4. Between flow velocities of 10 and 20 m·h^{-1} diffusivity was practically constant and close to the 0.7. This trend is related with biofilm heterogeneity. It is highly accepted that biofilms are composed by a matrix of cells and extracellular polymeric substances, and a large proportion of water (Hinson and Kocher 1996). Different studies (Horn and Morgenroth 2006) have suggested that due to this structure, solute transport in biofilms is the result of diffusion through the denser aggregates and convective transport

within pores and water channels. Therefore the approach which considers a homogeneous diffusion through biofilms, must take into account the two contributions to mass transport.

At low flow conditions, mass transport in biofilms was controlled by diffusion, as can be seen in Figure 3, where biofilm diffusivities below 10 $m \cdot h^{-1}$ were lower than in the rest of the range. On the other hand, at liquid velocities above 20 $m \cdot h^{-1}$ the phenomenon which dominates mass transport in biofilms was convection, with biofilm diffusivities even higher than water diffusivity, and close to typical convective mass transport coefficients. However, high liquid velocities are not recommended since an increase of shear stress could cause biofilm detachment. Between these two velocities, mass transport took place from the combination of both transport phenomena, with biofilm diffusivities practically constant over the range.

Biofilm diffusivity correlations

Comparison to experimental correlations

Experimental results of biofilm diffusivity presented in Figure 2a, were compared with the different correlations available for biofilm diffusivity calculation as function of biomass density.

Figure 2b reveals that experimental results of Figure 2a differed significantly with some of the correlations, especially in high range of concentrations. The largest deviations were observed respect to Zhang and Bishop equation. This equation was constructed from a theoretical study which approximated biofilm to a porous catalyst, and uses a developed catalyst model to describe mass transport through biofilms. Taking this into account, Zhang and Bishop equation can only be used as a rough approach for biofilm modeling. Compared to the measured data, Fan equation overestimates the diffusivity of biofilms denser than 40$gVSS \cdot L^{-1}$. The explanation of these differences is that Fan equation uses diffusivity results obtained in biomass granules at highest density range. These results highlight the necessity of having consistent experimental data for the development of diffusivity correlations. Hinson and Kocher Equation presented the same deviation observed for Fan equation. Hinson and Kocher developed their correlation by correcting Fan Equation with experimental diffusivities until a biomass density of 60$gVSS \cdot L^{-1}$. On the other hand, Horn and Morgenroth Equation, developed from experimental biofilm diffusivity estimations, fitted well with the results presented in the current paper. The only differences observed were in the lowest biomass density range, where Horn and Morgenroth correlation overestimates biofilm diffusivities, with biofilm diffusivities higher than water diffusivity. As Horn and Morgenroth described, this deviation was caused by the large scatter of the data used.

Multi-variable correlation

In order to solve the reliability problems shown by the different diffusivity correlations presented in Figure 2b, a new model for mass transport estimation in biofilms was developed. The biofilm diffusivities quantified at different biomass densities and liquid flow velocities, as the most influential parameters affecting diffusivity within biofilms. Figure 2a and Figure 3 respectively, were used in the development of multi-variable correlation. This study was carried out by two different approaches, a linear multi-variable model, Figure 4a, and a general multi-variable model, Figure 4b.

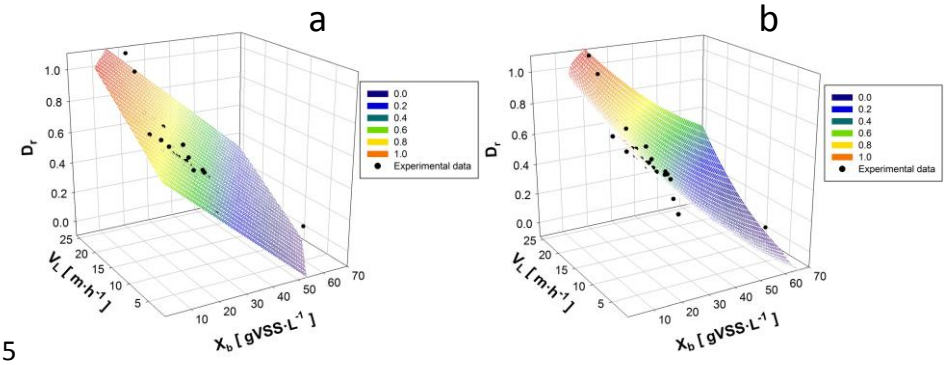

Figure 4. Two different approaches for biofilm diffusivity estimation from liquid flow velocity and biofilm density. a) Linear multi-variable model, and b) general multi-variable model.

Figure 4 shows as both models predicted well the biofilm diffusivity over the studied biomass range, without differences between them. However slight differences between both correlations were observed in diffusivity estimations through the liquid flow velocity range. As can be seen, for the lower velocities, below 10m·h^{-1}, the general equation described fairly worse the experimental diffusivity trend.

The diffusivity correlation obtained from the linear multi-variable fit is given in Eq. 3. Moreover, the correlation resulted from the general multi-variable adjustment is presented in Eq. 4.

$$D_r = 0.658 + 0.0258 \cdot V_L - 0.0147 \cdot X_b \quad R^2 = 0.9323 \qquad \text{Eq. 3}$$

$$D_r = 0.934 - 0.0227 \cdot X_b + 8.962 \cdot 10^{-4} \cdot V_L^2 + 1.057 \cdot 10^{-4} \cdot X_b^2 \quad R^2 = 0.9497$$

Eq. 4

As can be observed in Eq. 4 and Eq. 5, the correlations coefficient of both models revealed that mathematically there are practically no differences between them.

CONCLUSIONS

In this study, DO mass transport within biofilms has been quantified using a novel microsensor and a dynamic oxygenation procedure. In addition, it has been demonstrated that oxygen diffusivity results could be used in the intrinsic biofilm diffusivity characterization. Using this procedure biofilm mass transport was studied in a range of operational conditions. It was observed that a biofilm diffusivity linearly decreases when biofilm density increase. Furthermore in biofilms with densities over 50 g VSS·L^{-1} mass transport is strongly limited. These results, which differed from the available diffusivity correlations, revealed that a comprehensive biofilm control, a large experimental study and a consistent theoretical approach are required in order to develop a reliable correlation for diffusivity estimation. With this goal, hydrodynamics effect on mass transport was also investigated, concluding that liquid phase velocity (over biofilm) clearly modifies mass transport within biofilm. Finally, the development of a novel correlation which also includes hydrodynamics effect on mass transport provides a more accurate approach to diffusivity study in biofilms.

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References

Beyenal H., Lewandowski Z. Water Res 34 (2000) 528–538.
Bishop P., Zhang T., Fu Y. Water Sci Technol 31 (1995) 143–152.
Bonilla D., Mallén M., de la Rica R., Fernández-Sánchez C., Baldi A. Anal Chem 83 (2011) 1726–31.
Bradford MM. Anal Biochem 72 (1976) 248–254.
Fan L., Wisecarver KD., Zehner BJ. Biotechnol Bioeng 35 (1990) 279–286.
Fu YC., Zhang TC., Bishop PL. Water Sci. Technol. (1994) 455–462.
Gabriel G., Erill I., Caro J., Gómez R., Riera D., Villa R., Godignon P. Microelectronics J 38 (2007) 406–415.
Guimera A., Gabriel G., Plata-Cordero M., Montero L., Maldonado MJ., Villa R. Biosens Bioelectron 31 (2012) 55–61.
Guimerà X., Moya A., Dorado AD., Villa R., Gabriel D., Gabriel G., Gamisans X. Appl Microbiol Biotechnol. 99 (2014) 55-56.
Hinson RK., Kocher WM. J. Environ. Eng. 122(1996) 1023–1030.
Horn H., Morgenroth E. Chem Eng Sci 61 (2006) 1347–1356.
Lewandowski Z., Beyenal H. Fundamentals of biofilm research, Boca Raton (2007) 452.
Matson J V., Characklis WG. Water Res 10 (1976) 877-885
Mora M., López LR., Gamisans X., Gabriel D. Chem Eng J 251 (2014) 111–115.
Mottola HA. Anal Chem 50 (1978) 94–98.
Moya A., Guimerà X., del Campo FJ., Prats-Alfonso E., Dorado AD., Baeza M., Villa R., Gabriel D., Gamisans X., Gabriel G. Microchim Acta. (2014) Article in Press.
Nguyen MT., Appan A., Tan DS., Tan SK. J Environ Eng 140 (2014) Article in Press.
Ning Y-F., Chen Y-P., Li S., Guo J-S., Gao X., Fang F., Shen Y., Zhang K. Anal Methods 4 (2012) 2242
Stewart PS. Biotechnol Bioeng 59 (1998) 261–272.
Zhang TC., Bishop PL. Water Res 28 (1994a) 2279–2287.
Zhang TC., Bishop PL. Water Res 28 (1994b) 2267–2277.
Zhou X-H., Liu J., Song H-M., Qiu Y-Q., Shi H-C. Environ Eng Sci 29 (2012) 466–471.