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Influence of the hydrodynamics on the biofilm formation by mass transport analysis

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Abstract

Biofilm are formed wherever there is some water in our environment: pipes, pipelines, tap water systems, air conditioning systems... Furthermore, the ecological and economical consequences are very important: energy losses, bacterial contamination, material deterioration. The aim of this work is to develop a new method to detect and monitor the biofilm formation. This method can also determine some mechanical properties of the biofilm. An application of this method is a realization of a biofilm sensor.

Biofilm is considered as an inert porous layer with respect to mass transport. In our experiment, the biofilm is grown on a gold electrode in natural seawater. Its thickness is determined by considering the oxygen diffusion limiting current measured for different rotation speeds on this electrode. Two different incubators are used during the biofilm development: one, with a laminar flow, permits the rotation of the electrode during the biofilm formation, and for the second, a tube is used under turbulent conditions during the biofilm formation. This experiment allows us to characterize the mechanical behavior (thickness, elasticity, rigidity) of the biofilm in function of different conditions of development.

Keywords: Biofilm; Deformation; Elasticity; Mass transport analysis; Oxygen reduction; Thickness

1. Introduction

Biofilm formation has numerous detrimental consequences: health risks (hospitals, tap water systems, climatisation systems...), reduced industrial performance (energy losses, increased heat transfer resistance, increased fluid frictional resistance...), and material deterioration (pipelines, port installations...) [1–4]. To reduce this problem, we must investigate the biofilm formation, and for that, it is necessary to use a sensor to detect them [5]. This will allow better control over the biofilm formation and damage.

Biofilms are generally composed of microbial cells and their products (extracellular polymers), in agreement with the amount of water contained ($>95\%$), which confer to them a very porous structure [6,7]. The distribution of microorganisms is not uniform with a microscopic point of view [8–10]. In multi-species biofilms, highly complex structures containing voids, connecting channels between these voids, and microbial clusters or layers were predominantly found [11]. With respect to mass transport, biofilms behave as an inert porous layer. And at the macroscopic scale, biofilms can be considered as layers of stagnant water on the material surface. By Electro-Hydro-Dynamical impedance [12,13], it was shown that the diffusion coefficient in the biofilm D_f is the same as the diffusion coefficient in the water D. The porosity of the biofilm is, therefore, close to 1, in agreement with the amount of water contained. Some techniques based on ex-situ measurements (not good methods, considering the amount of water of the biofilm) can be used for the biofilm thickness determination if the biofilm is thick enough [14,15], but the methods based on light microscopy allow measurement of biofilm thickness of tens of micrometers or more. The novelty of the method used in this work is that it allows an

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in-situ thickness measurement of very thin biofilms and the biofilm monitoring on line [16].

Mass transfer to a rotating electrode has been studied in the case of an interface covered by a porous layer [17,18]. The method was based on the analysis of tracer (oxygen or ferricyanure [5]) reduction current under total mass transport control (diffusion plateau of the electroactive species). The layer thickness δ_f is the characteristic parameter under investigation.

The aim of this paper is to show the evolution of the biofilm obtained in natural seawater, in function of its mechanical properties (thickness, elasticity and deformation). The biofilm is characterized by the limiting current analysis of the oxygen reduction.

2. Mass transport through a porous layer

When reacting species are oxidized or reduced in a fast reaction at a metallic electrode coated by a porous layer, their concentration gradient is distributed in two components: in the porous layer, the mass transport is controlled by molecular diffusion, in the electrolyte solution, it is controlled by convective diffusion [18] (Fig. 1). The metal layer interface is assumed to be uniformly reactive.

For the steady-state measurements, at a given potential corresponding to the tracer diffusion plateau, a current density is measured as a function of the electrode rotation speed. The steady-state current can be analytically calculated as follows, according to the Levich theory [19] and Deslouis et al. [18]:

$$I = I_0 + \frac{1}{I_f^{-1} + I_L^{-1}} \quad (1)$$

with:

$$I_f = \frac{nFD_f c_\infty S}{\delta_f} \quad (2)$$

$$I_L = K^* \Omega^{0.5} \quad (3)$$

$$K = 0.62\, nFSD^{2/3}\nu^{-1/6}c_\infty \quad (4)$$

and where I_0 is a non-diffusional current, corresponding for example to the hydrogen reduction, D is the diffusion coefficient in the electrolyte, D_f is the diffusion coefficient in the film (and $D \cong D_f$ [12,17]), c_∞ the concentration of the electroactive species in the bulk solution, δ_f is the porous layer thickness, K the Levich coefficient, F is the Faraday constant ($F = 96485$ Cb), n is the number of electrons ($n = 4$ for the oxygen), S is the active electrode area ($S = 0.785$ cm^2), ν is the kinematic viscosity (10^{-2} cm^2 s^{-1}) and Ω is the disk rotation speed.

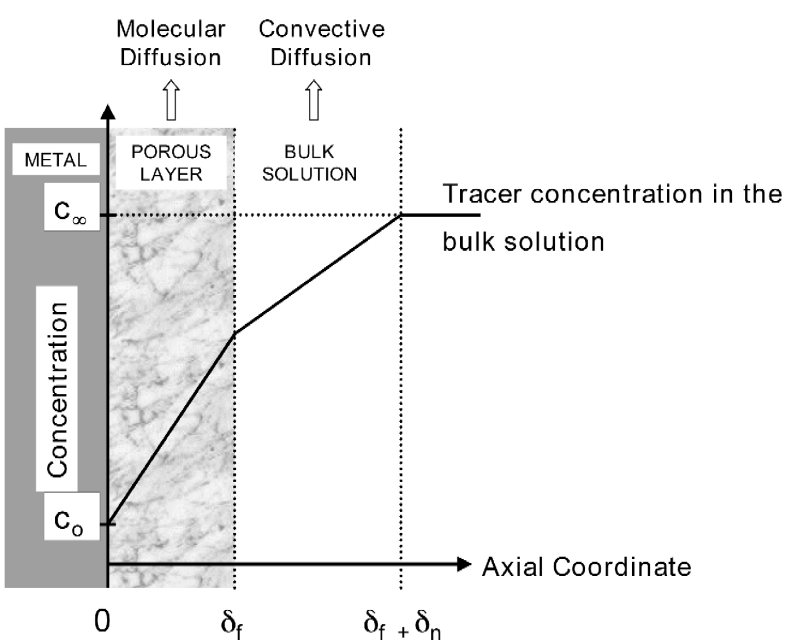

Fig. 1. Variation of tracer concentration versus axial coordinate, where δ_f is the porous layer thickness, δ_n the diffusion layer thickness, c_∞ the concentration of the electroactive species in the bulk solution and c_0 the concentration of the electroactive species at the interface porous layer/electrode.

3. Experimental techniques

In the case of the experiments, the porous layer consists of a biofilm developed on an inert gold electrode (10 mm diameter) during the immersion time in natural seawater from the Atlantic Ocean (the Brest roadstead, France). Before immersion, the electrodes were polished using silicon carbide (grade 4000) and then cleaned ultrasonically in distilled water.

For the steady-state measurement, the disk electrode was rotated by a DC motor system and the current was picked up by mercury contacts. The motor speed was controlled with a servo system and measured by means of a tachometer.

The oxygen was used as tracer. The oxygen tracer has the advantage of being naturally present in the medium. The oxygen diffusion coefficient is 1.6×10^{-5} cm^2 s^{-1}, its concentration was 2.2×10^{-4} M, and was determined by the polarographic method during the measurements.

Two sorts of incubators were used for the biofilm development in seawater, renewed continuously (Table 1). One allowed an electrode rotation of 300 rpm during all immersion (before and after the measurement) and the laminar flow was controlled during the biofilm growth (Fig. 2A). With the first incubator, the rotation speed varies only during the measurement, this incubator is also the electrochemical cell. The second incubator permitted a biofilm development in a tube with a controlled turbulent flow at around 0.5 m s^{-1} (before and after the measurement) (Fig. 2B). For the steady-state measurement only, the electrodes were moved in an electrochemical cell, where the laminar flow could be controlled.

During the steady-state measurements, the electrode rotation speed range was increased for 100 to 1100 rpm by steps of 50 mV. For each curve, the data points were obtained with increasing velocity (100 to 1100 rpm) and with decreasing velocity. As the two curves were almost identical, only one set of data is presented for clarity. The electrode was polarized -900 mV/SCE on the oxygen limiting diffusion plateau. The limiting current was the same before and after the electrode rotation speed change, without a hysteresis. Thus, if the biofilm thickness recovers to its initial value in a short time, the change of film thickness could indeed be attributed to elastic behavior.

Table 1
Characteristic parameters in seawater in the Atlantic Ocean

pH	8.0–8.3
[O_2]	7 mg l^{-1}
Salinity (NaCl, KCl in majority)	34 g l^{-1}
Turbidity	0.5–5 NTU
Conductivity	40–54 mS cm^{-1}
Bacterial density	10^2–10^3 bact ml^{-1}
Thermostatic temperature	23 ± 2°C
Main re-liveable bacteria (not exhaustive)	*Pseudomonas* sp., *Pseudomonas, Acinobacter*, *Vibrio vulnificus*

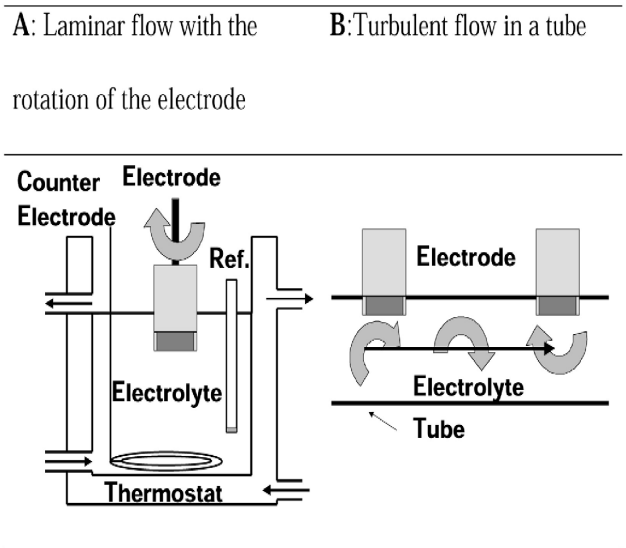

Fig. 2. Incubators representation during biofilm development.

4. Results and discussion

4.1. Determination of δ_f for a biofilm with oxygen tracer

At time $t = 0$, no biofilm is present at the interface and so $I_f^{-1} = 0$. And hence, the recorded current is according to the Levich Law (Eq. (1)):

$$I(0) = I_0 + I_L . \quad (5)$$

Usually for the mass transport analysis through a porous layer, the reciprocal steady-state current is recorded versus the reciprocal square root of the electrode rotation speed in the Koutecky–Levich coordinates (Fig. 3). For the determination of the biofilm thickness, in the case of the oxygen tracer, the non-diffusional current I_0 existed. The determination of I_0 by analysis of the Levich curve at $t = 0$ (Fig. 4) yielded the value of 15 μA. The non-diffusional current was, in the present case, the hydrogen reduction current, and I_0 could be assumed to be independent of time and of the biofilm growth, since the electroactive surface did not change. The corresponding curve is parallel to the Levich Law curve (Fig. 3).

For a given time, the current increased with the electrode rotation speed according to the Levich Law, but for a given rotation speed, the current decreased with time due to biofilm formation. For a time t $(t > t(0))$, $I_f^{-1} \neq 0$. Then with Eq. (2) the thickness of the porous layer is given by:

$$\delta_f = nFD_f c_\infty \left(\left(I(t) - I_0 \right)^{-1} - \left(I(0) - I_0 \right)^{-1} \right). \quad (6)$$

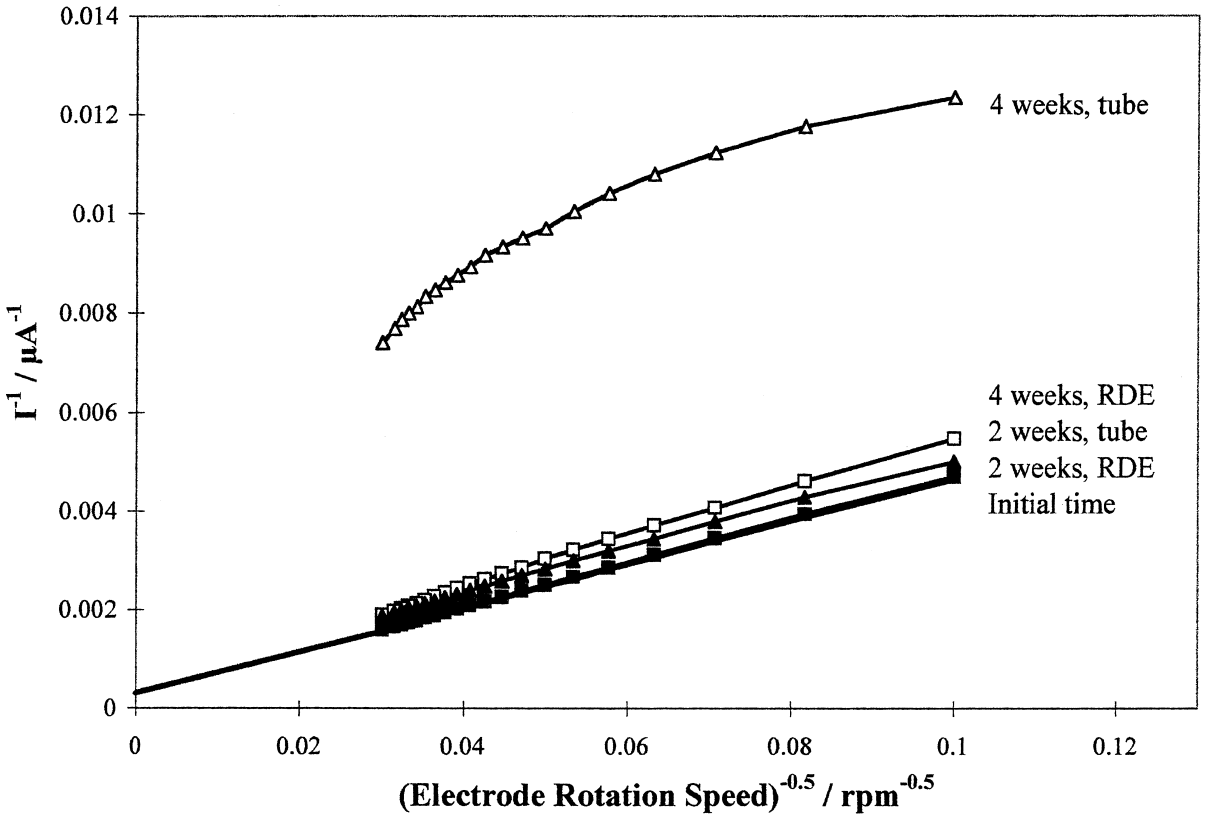

Fig. 3. Inverse current evolution with the electrode rotation speeds at different immersion times (— initial, □ ■ 2 weeks and △ ▲ 4 weeks) in two sorts of incubators (black symbols RDE in laminar flow and white symbols tube in turbulent flow) with the oxygen tracer on a gold electrode in seawater.

For the two flow conditions studied, on the gold electrode, the current decreased with the immersion time because of the presence of a biofilm (Fig. 3). This approach to determine biofilm thickness is new because until now

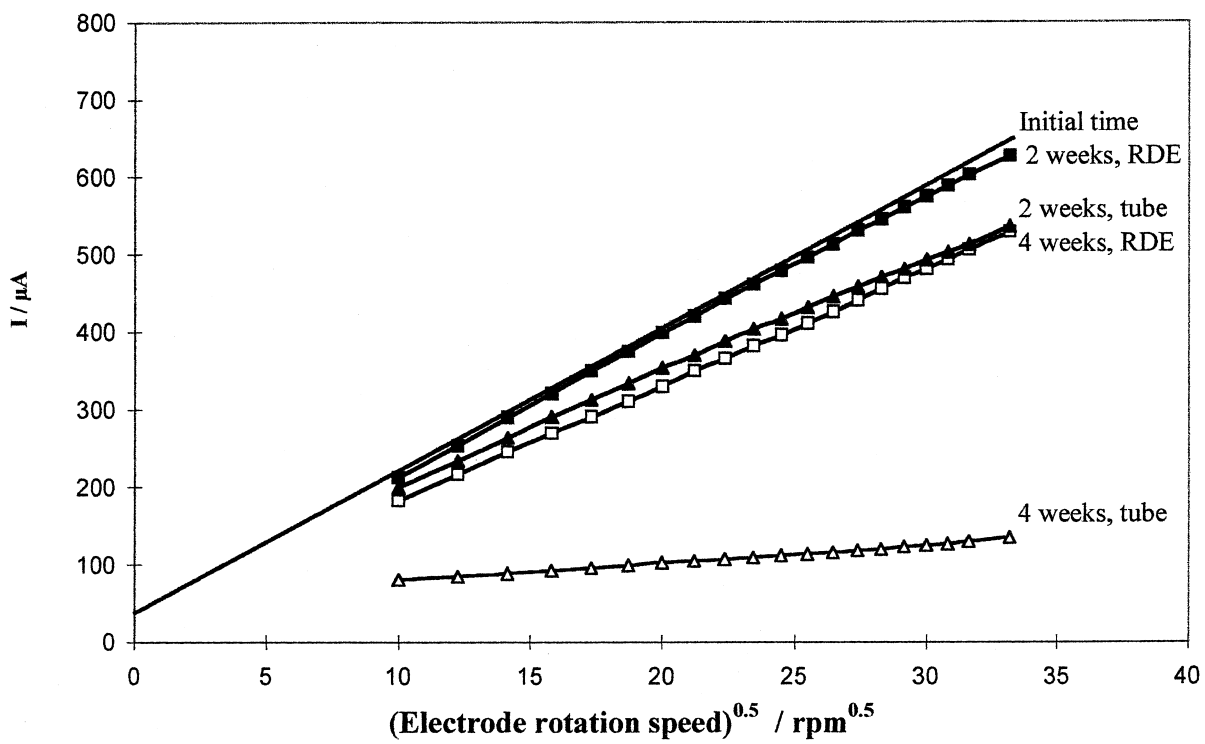

Fig. 4. Current evolution with the electrode rotation speeds at different immersion times (— initial, □ ■ 2 weeks and △ ▲ 4 weeks) in two sorts of incubators (black RDE in laminar flow and white tube in turbulent flow) with the oxygen tracer on a gold electrode in seawater.

the porous layer was considered to be a solid one with a thickness independent of the flow [12,13,17], in the present case, it is necessary to consider an elastic and flexible behavior for the biofilm. The term "elastic" may be used because the same current, and thus the same thickness, is obtained by increasing or decreasing the rotation speed. The measurement at high rotation speeds was not performed to avoid a mechanical degradation of the biofilm. The critical speed was obtained when after return to low speeds, the thickness was not the same. The transient response could not be used to extract a time constant for the elastic behavior because we do not know the model of biofilm development in all the case. In fact, the composition and physical properties of biofilm may depend on the conditions under which it is grown, because of the different elasticity of the film.

At two immersion weeks, in the incubator A, a small modification of the current was obtained, allowed biofilm thickness determination with Eq. (6) (Figs. 4 and 5). In the incubator B, the oxygen reduction current was decreased in an important way and a thickness of 3 μm is measured on the electrode (Fig. 5). This way of following the thickness indicates that in both case, the biofilm is little flexible and that the influence of the hydrodynamics on it is not very pronounced.

After 4 weeks of immersion, the reduction current is significantly lower on the electrode in the tube than on the electrode in rotation (Figs. 4 and 5). The biofilm thickness varies between 8 μm at 100 rpm and 3 μm at 1100 rpm on the electrode in rotation; whereas the thickness change between 80 and 53 μm for rotation speeds of 100 and 1100 rpm for the electrode in immersion in a tube during the biofilm development (Fig. 6). In the first case, the thickness, weak, has decreased of more of half, corresponding to a flexible biofilm, while in the other case, the thickness decrease is not so important with the rotation speed (but still almost half), corresponding to a biofilm less flexible. The water being relatively incompressible, the decrease of biofilm thickness does not change the value of diffusion coefficients.

4.2. Biofilm deformation

In order to know the influence of the rigidity during the biofilm formation, the deformation is represented in function of the electrode rotation speed (Fig. 7). This deformation is obtained by the difference between the thickness at a given rotation speed and the thickness at 100 rpm.

For the biofilm obtained in the incubator A (laminar flow), the deformation change with the electrode rotation speed, but in a non-constant manner. The flexibility is dependent of the electrode rotation speed. For the biofilm developed in the incubator B, the deformation is constant with the electrode rotation speed. The flexibility is independent of the rotation speed. The biofilm mechanical properties are different in function of the development conditions.

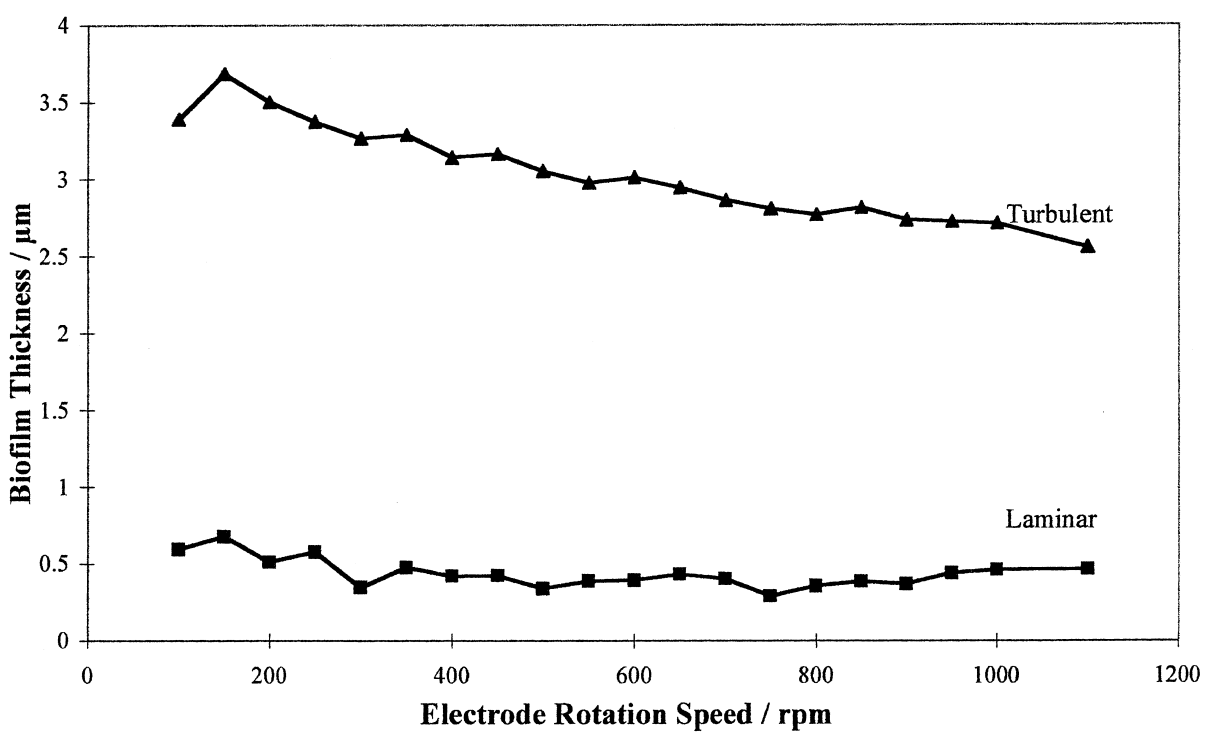

Fig. 5. Biofilm thickness evolution with electrode rotation speeds at 2 weeks of immersion in a tube with a turbulent flow (▲) and on a RDE with a laminar flow (■).

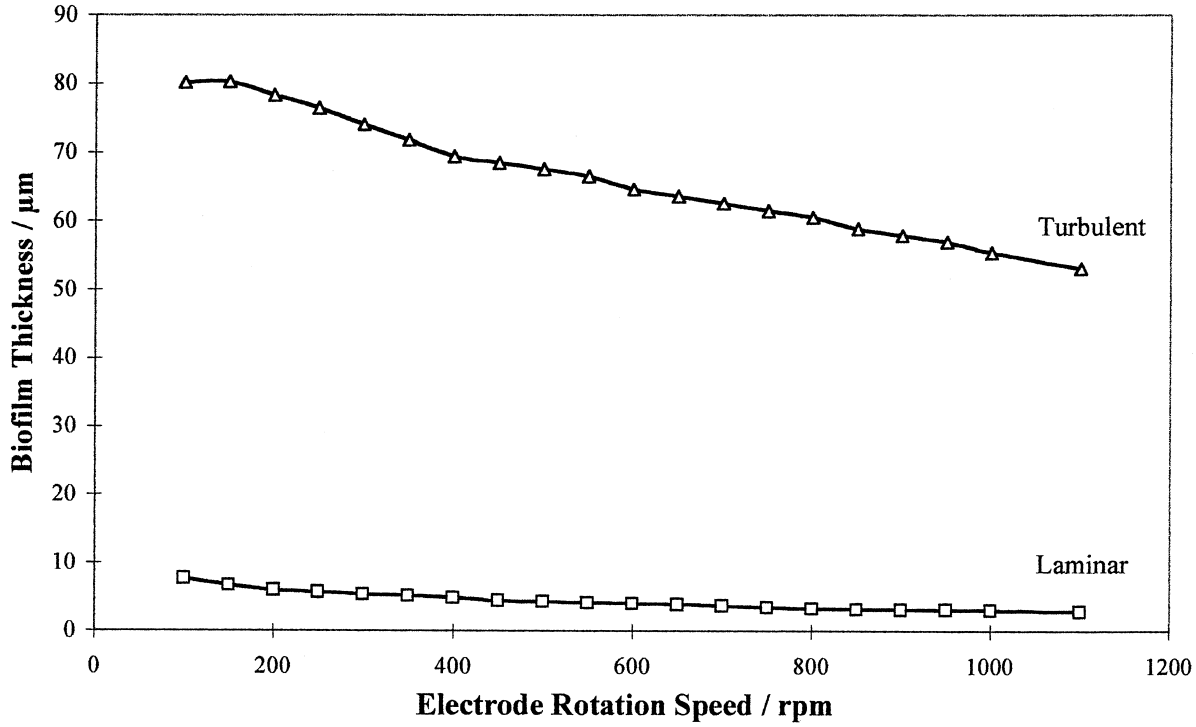

Fig. 6. Biofilm thickness evolution with electrode rotation speeds at 4 weeks of immersion in a tube with a turbulent flow (△) and on a RDE with a laminar flow (□).

For the high speeds (between 800 and 1100 rpm), the deformation is two times more important in the case of the biofilm developed in the incubator A (laminar flow with rotation of the electrode) than in the case of the incubator

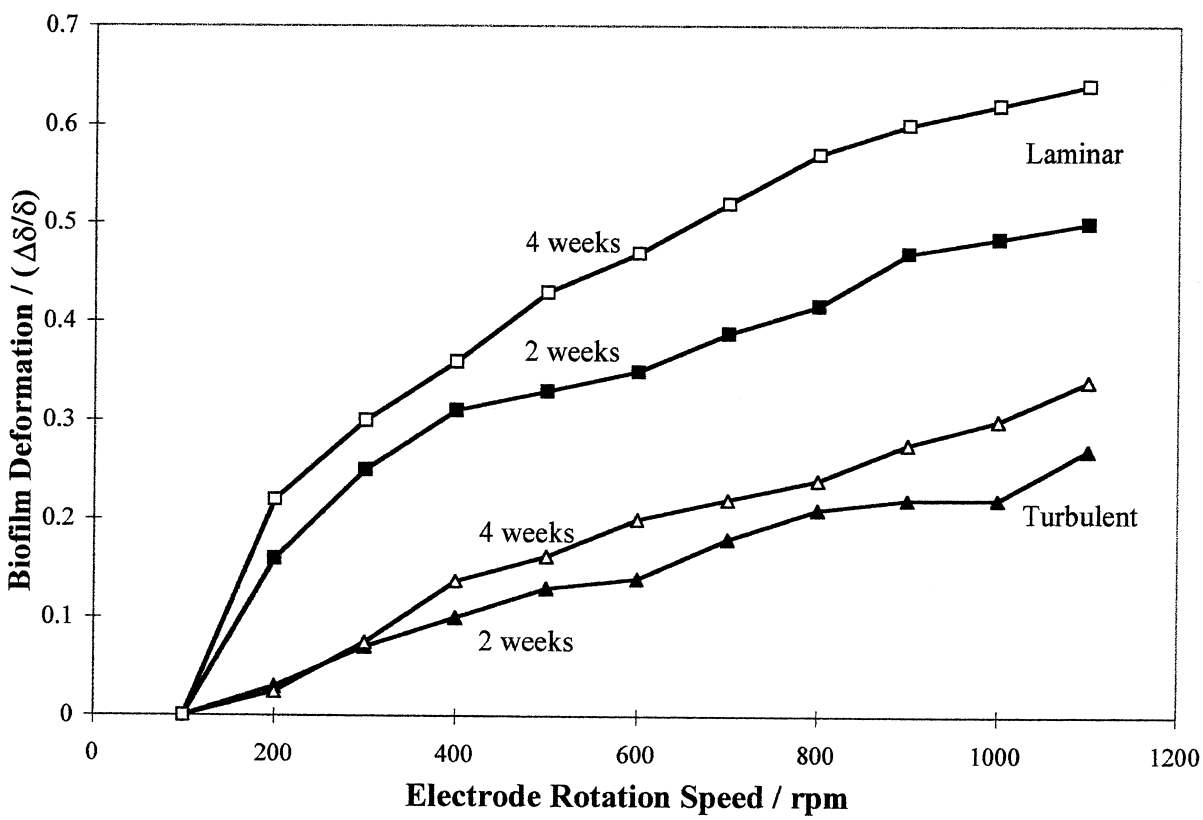

Fig. 7. Biofilm deformation evolution with electrode rotation speeds and with exposition types (laminar flow with rotation of the electrode and turbulent in a tube) after.

B biofilm (turbulent flow in a tube), and this moreover at 2 weeks or at 4 weeks.

These results suggest that the biofilm structure is very dependent of the formation conditions: laminar or turbulent flow, exposition place, immersion times… This easy method of determination of biofilm thickness gives some new information about the structure, according to Costerton [8,9], Costerton et al. [10], Videla [11], Beardwood [20], De Beer et al. [21] and Melo and Bott [22].

5. Conclusion

The aim of this work is to show the influence of biofilm development conditions on the mechanical properties. For that, a new electrochemical method is used to follow the elasticity and the flexibility of the biofilm. On one hand, the biofilm is elastic, whatever the development conditions (laminar flow with electrode rotation or turbulent flow in a tube). The thickness is the same before and after the mechanical strain (rotation speed). On the other hand, the deformation depends on the development conditions and the mechanical properties of the biofilm are different. This new approach can be performed to detect and monitor the biofilm formation with a sensor.

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