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Impact of electrode micro- and nano-scale topography on the formation and performance of microbial electrodes

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A B S T R A C T

From a fundamental standpoint, microbial electrochemistry is unravelling a thrilling link between life and materials. Technically, it may be the source of a large number of new processes such as microbial fuel cells for powering remote sensors, autonomous sensors, microbial electrolyzers and equipment for effluent treatment. Microbial electron transfers are also involved in many natural processes such as biocorrosion. In these contexts, a huge number of studies have dealt with the impact of electrode materials, coatings and surface functionalizations but very few have focused on the effect of the surface topography, although it has often been pointed out as a key parameter impacting the performance of electroactive biofilms.

The first part of the review gives an overview of the influence of electrode topography on abiotic electrochemical reactions. The second part recalls some basics of the effect of surface topography on bacterial adhesion and biofilm formation, in a broad domain reaching beyond the context of electroactivity. On these well-established bases, the effect of surface topography is reviewed and analysed in the field of electroactive biofilms. General trends are extracted and fundamental questions are pointed out, which should be addressed to boost future research endeavours. The objective is to provide basic guidelines useful to the widest possible range of research communities so that they can exploit surface topography as a powerful lever to improve, or to mitigate in the case of biocorrosion for instance, the performance of electrode/biofilm interfaces.

1. Introduction

A huge number of bacteria have revealed their capability to perform extracellular electron transfer with electrodes (Logan and Regan, 2006; Koch and Harnisch, 2016). Two main strains, *Geobacter sulfurreducens* (Bond and Lovley, 2003; Lovley et al., 2011) and *Shewanella oneidensis* (Ringeisen et al., 2006; Fredrickson et al., 2008) have been widely used as model organisms for basic investigations, because of their early discovery as electroactive strains and their high performance. In addition, *G. sulfurreducens* has the capability to achieve both anodic and cathodic electron transfers (Bond and Lovley, 2003; Dumas et al., 2008a; Soussan et al., 2013). However, multi-species microbial communities are most often used as the inoculum when the objective is to design microbial anodes to be implemented in microbial electrochemical processes. Environmental samples coming from marine or lake sediments (Reimers et al., 2001; Girguis et al., 2010; Zabihallahpoor et al., 2015; Grattieri and Minteer, 2018), wastewater, sludge from treatment plants (Fornero et al., 2010; Kokko et al., n.d.), and soils (Cercado Quezada et al., 2013; Doyle and Marsili, 2015) have been particularly used as multi-species inocula and many others can be

implemented (Chabert et al., 2015).

The majority of studies have been devoted to microbial anodes (Pham et al., 2009; Wagner et al., 2010; Kumar et al., 2013; Lu et al., 2015). Microorganisms oxidize organic compounds to sustain their metabolism and the resulting low energy electrons are released to the electrode through the biofilm (Fig. 1). A smaller number of studies have dealt with microbial cathodes, mainly for oxygen reduction (Bergel et al., 2005) and, more recently, CO₂ reduction (Rabaey et al., 2011; ElMekawy et al., 2016; Bajracharya et al., 2017).

Microbial electrodes have been considered as the possible source of a large number of new processes (Wang and Ren, 2013; Schröder et al., 2015; Bajracharya et al., 2016). Some exaggeratedly enthusiastic forecasts about the future of microbial electrochemical technologies are open to question (Blanchet et al., 2015; Olliot et al., 2016) but microbial electrodes should, nevertheless, be at the core of many innovative applications, provided that suitable objectives are chosen. For instance, microbial fuel cells may be appropriate energy production systems when low power is sufficient (Shleev et al., 2015), such as for powering remote sensors (Dewan et al., 2014) and designing autonomous sensors (Di Lorenzo et al., 2009; Pasternak et al., 2017). Simplifying the

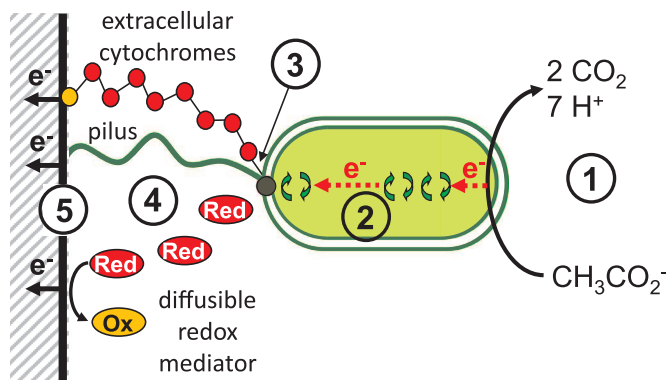


Fig. 1. Scheme of electron transfer mechanism through an electroactive anodic biofilm, adapted from (Strycharz et al., 2011). 1) Diffusion of the substrate to and of the products from the microbial cells, 2) metabolic redox reactions, 3) electron transfer from the cell to extracellular redox compound, 4) electron transport through the biofilm matrix, 5) electron transfer to the electrode surface.

equipment to design low-cost devices that do not require attendance, such as the electro-microbial snorkel (Erable et al., 2011; Matturro et al., 2017), or focusing on specific environments, such as hypersaline media (Rousseau et al., 2013; Carmona-Martinez et al., 2015; Grattieri and Minteer, 2018) may also open up promising horizons. Furthermore, microbial electrodes have led to fundamental discoveries on the electrochemical link between living organisms and materials (Borole et al., 2011; Shi et al., 2016; Kumar et al., 2017), which may be involved in many natural processes, such as anaerobic digestion (Kato et al., 2012; Liu et al., 2012) and microbially influenced corrosion (Beech and Sunner, 2004; Mehanna et al., 2009a; Kip and van Veen, 2015).

Very many studies have focused on electrode materials and coatings (Liu et al., 2007; Wei et al., 2011; Guo et al., 2013; Santoro et al., 2015; Xie et al., 2015). Actually, the biofilm/electrode interface has been widely investigated from the standpoint of surface chemistry (Lowy et al., 2006; Rosenbaum et al., 2007; Scott et al., 2007; Kumar et al., 2013; Du et al., 2014), with many attempts at surface functionalization (Erable et al., 2009a; Picot et al., 2011; Lapinsoinière et al., 2013; Zhao et al., 2013; Ding et al., 2015; Li et al., 2017). Surprisingly, the topography of the biofilm/electrode interface has rarely been at the heart of the studies, although it has often been pointed out as a key parameter for the efficiency of microbial electrodes (Peng et al., 2010; Sun et al., 2010; Fan et al., 2011; Pons et al., 2011a; Pocaznoi et al., 2012a). To the best of our knowledge, the studies dealing with the impact of the electrode topography on electroactive biofilms have never been reviewed so far. Such a review is the purpose of the present article, with the objective of trying to extract general guidelines and suggesting, if relevant, useful directions for future research.

The first part of the present article recalls the basics concerning the effect of surface topography on bacterial adhesion and biofilm formation. It gives an overview of the overall knowledge that has been established in a general field, out of the context of electroactivity. The second part deals with the impact of electrode topography on abiotic electrochemical reactions, i.e. in the absence of microbially-related phenomena. On these two well-established bases, the studies that deal with surface topography in the field of electroactive biofilms are then reviewed and analysed.

One objective is to see whether general trends can be extracted and whether or not they follow the knowledge previously established for non-electroactive biofilms and in the field of abiotic electrochemistry. Secondly, this cross-cutting analysis points to exciting questions that should be addressed in order to encourage future research endeavours in the most relevant directions. Among various suggestions, the main conclusion that should be kept in mind is the necessity to take the considerable effect of surface topography into better consideration

when analysing any experimental data related to electron transfer at biofilm/electrode interfaces in the future.

We hope that this review will offer useful guidelines for exploiting surface topography as a lever to improve the performance of electroactive biofilms. Furthermore, even though the main current trend is towards the design of electroactive biofilms for microbial electrochemical processes, it should not be forgotten that the same interfaces can be at the heart of microbially influenced corrosion (Mehanna et al., 2009a, 2009b). In this field, the same fundamental knowledge is fully relevant to fight against corrosion by mitigating interfacial electron transfers. In both cases, this review article has been written with the objective of giving the widest possible range of research communities some helpful information for further basic and technological thinking.

2. Basics of surface topography

2.1. Macro-, micro- and nano-roughness

Each surface, even the smoothest one, contains irregularities, which can occur at macro-, micro- and nano-scale. This review focuses the micro- and nano-scale surface topography, because this is the range of sizes that can impact the mechanisms of bacterial adhesion, biofilm formation and electron transfer reactions. The surface waviness, also referred as macro-roughness, is not taken into account in this review article. Actually, it may affect the global performance of microbial electrochemical systems, by modifying the surface area that is available for microbial colonization, but it is not thought to influence the mechanisms of electroactive biofilm formation and operation.

2.2. Characterization of surface topography: R_a is not perfect but is useful

Any surface is perturbed by asperities and valleys at micrometre and nanometre scales. The surface roughness profile is commonly characterized by the arithmetic mean roughness value (R_a) measured along a line. R_a is defined as the average absolute deviation of the roughness irregularities from the mean line (Table 1). For example, this means that a surface with R_a of 1 μm presents peaks and valleys 1 μm above and 1 μm below the mean line on average. Since it is one of the easiest to measure, R_a has become a standard parameter. Nevertheless, it depicts the surface topography only vaguely (Donoso et al., 2007), since various surface profiles can present the same R_a value. A large variety of parameters have been described in the literature to better characterize surface topography according to the field of study (Bharat Bhushan, 2000; Stout, 2000; Webb et al., 2012) (Table 1).

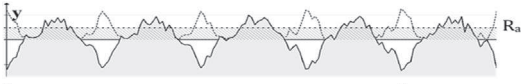
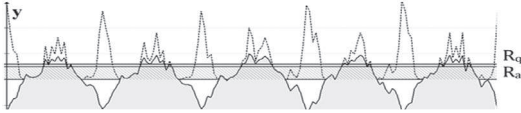


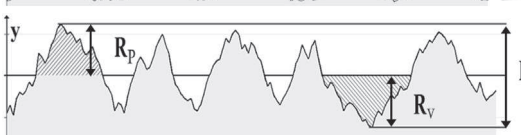
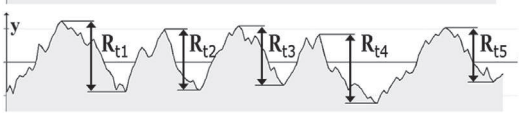
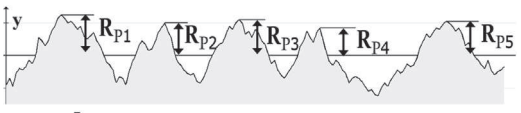
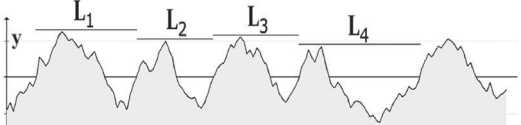
Several parameters have been based on the roughness profile (Table 1), i.e. the roughness along a line:

- skewness (R_{sk}), which measures the symmetry between peaks and valleys,
- kurtosis (R_{ku}), which measures the sharpness of surface,
- root mean square surface roughness (R_q),
- maximum peak to valley height (R_t or R_{max}),
- maximum peak height (R_p),
- maximum valley depth (R_v),
- average peak to valley height (R_z),
- average peak-to-mean height (R_{pm}),
- and mean width of the roughness elements (RS_m).

Other parameters have been based on an analysis of the two-dimensional roughness, instead of the roughness profile along a line. The so-called spatial parameters are: root mean square area roughness (S_q), summit density (S_{ds}), developed area ratio (S_{dr}), ten-point average roughness (S_z), skewness (S_{sk}), texture aspect ratio (S_{tr}) and bearing ratio (t_p) as proposed by Crawford et al. (2012).

The (S_i) parameters based on surface analysis result in better characterization of the surface shape and organization than those based on

Table 1
Roughness parameters recommended to describe a topographical profile.

Symbol	Parameter	Description	Formula	Schematic meaning
R_a	Roughness average	Average absolute deviation of the roughness irregularities from the mean line	$R_a = \frac{1}{n} \sum_{i=1}^n y_i $	
R_q	Root mean square	Arithmetic mean of the squares of deviation of the roughness irregularities from the mean line	$R_q = \sqrt{\frac{1}{n} \sum_{i=1}^n y_i^2}$	
R_{sk}	Skewness	Symmetry of the roughness in terms of peaks and valley	$R_{sk} = \frac{1}{nR_q^3} \sum_{i=1}^n y_i^3$	
R_{ku}	Kurtosis	Sharpness of the roughness defined as deviation from the mean line	$R_{ku} = \frac{1}{nR_q^4} \sum_{i=1}^n y_i^4$	
R_t or R_{max}	Maximum peak to valley height	Amplitude of the roughness between the maximum peak R_p and maximum valley R_v	$R_t = R_p - R_v$ $R_t = \max_i y_i - \min_i y_i$	
R_z	Average peak to valley height	Average of the R_t on n small profile lengths	$R_z = \frac{1}{n} \sum_{j=1}^n R_{tj}$	
R_{pm}	Average peak height	Average of the R_p on n small profile lengths	$R_{pm} = \frac{1}{n} \sum_{j=1}^n R_{pj}$	
RS_m	Mean peak spacing	Mean width of the roughness profile elements	$RS_m = \frac{1}{n} \sum_{j=1}^n L_j$	

the analysis of one-dimensional roughness profiles (R_i). However, spatial parameters are more complex to determine and require specific analytical equipment. They have consequently been largely less implemented than the parameters based on profile roughness, among which R_a is almost always used.

3. Impact of surface topography on electrochemical reactions

3.1. Micro-roughness and electrochemical reactions

The current lines are perpendicular to the electrode surface, so they can be severely disturbed locally by the peaks and valleys created by surface micro-roughness (Gamburg and Zangari, 2011). Moreover, it is recognized that the micro-roughness of the surface enhances electrode performance by virtue of both the larger active surface area it creates and the increased local mass transfer due to micro-turbulence (Gabe et al., 1998).

Because of their importance, the effects of micro-roughness have been the subject of many theoretical studies aimed at modelling the electrode surface topography. Two different approaches have been investigated. On the one hand, roughness can be assimilated to well-defined micro-structures. Electrodes covered with arrays of bell-shaped or conical micro-peaks (Menshykau and Compton, 2009; Popov et al., 2010), cylindrical microelectrodes (Dickinson et al., 2008), spherical and hemispherical micro- and nano-particles (Streeter et al., 2007; Streeter and Compton, 2007; Popov et al., 2010) and micro-peaks with arbitrary sinusoidal shapes (Menshykau et al., 2008) have thus been

modelled. Roughness has also been modelled as an interfacial porous layer with defined pore shape and size (Real et al., 1992). The development of many techniques such as lithography, nano-printing and plasma etching, which allow micro-/nano-patterned electrode surfaces to be produced (Sánchez-Molas et al., 2012) or nano-/micro-particles to be deposited on electrode surfaces (Streeter et al., 2007), have given accurate experimental supports. It has thus been possible to refine the theoretical approaches by confrontation with accurate experimental data.

On the other hand, attempts have also been made to approach electrode surface roughness by means of random models. The objective was to establish global formalisms to characterize the electrode topography. The fractal concept has been implemented in several papers (Nyikos and Pajkossy, 1986; Pajkossy and Nyikos, 1989a, 1989b; Rammelt and Reinhard, 1990; Parveen and Kant, 2016). Other similar attempts have been made to define global parameters that could introduce the effect of the electrode roughness into electrochemical equations for non-fractal surfaces (Kant, 1993, 2010; Kant and Rangarajan, 1995; Sharma et al., 2017).

These different modelling approaches have mainly dealt with micro-roughness but they can also be applied to nano-roughness (Streeter et al., 2007; Streeter and Compton, 2007; Kant, 2010). Both the experimental and theoretical approaches have shown significant effects of the surface roughness on the current provided by electrodes. Obviously, roughness increases the active surface area and increases thus the current density related to the electrode projected surface area. It has also been shown that roughness increases the transport-limiting current

by acting on the local diffusion profile (Kant and Rangarajan, 1994; Streeter and Compton, 2007). It also modified the conditions of adsorption redox compounds on the electrode surface (Menshykau and Compton, 2009) and impact the double layer capacity (Douglass Jr. et al., 2008). The impact of surface roughness on abiotic electrochemistry has been judged so important that a recent article has stated that: "...not accounting for roughness in data analysis may cause errors in estimation of composition, diffusion coefficient, improper assignment of electrode mechanism, and so forth" (Parveen and Kant, 2016).

To the best of our knowledge, this vast theoretical basis developed for abiotic electrochemistry has not yet been exploited to investigate the impact of roughness on electroactive biofilms, although it may directly affect the step 5, or even possibly step 4, of the electron transfer chain (Fig. 1). Tapping into these studies would no doubt help the research community to make significant advances in understanding and improving microbial electrodes.

3.2. Nano-roughness and electrochemical reactions

Borisova and Ershler have reported pioneering work on the influence of nano-scale roughness on the double-layer capacitance (Borisova and Ershler, 1950). Capacitance dispersion decreased as the surface became flatter. Many studies then confirmed that capacitance dispersion was mainly of geometrical origin (De Levie, 1965; Scheider, 1975; de Levie, 1989). Since this discovery, surface roughness has been known to have a positive correlation with the electrical capacitance of the surface (Albina et al., 2006). It has also been stated that electrical conductance is reduced by deposited films, which decrease the nano-roughness. This effect has been observed for thin films, tens of atoms thick (Ke et al., 2009). Nano-roughness is also known to impact molecular adsorption on electrode surfaces (Pfeifer et al., 1989). The concentration of adsorbed species tends to be smaller and the adsorption process slower on a rough surface than on a smooth one, because of the perturbation of the molecular arrangement caused by the roughness. Adsorption can lead to great variations in the interfacial properties (Bockris et al., 2008), especially in the electrode double-layer capacitance (Douglass et al., 2008).

4. Impact of surface topography on cell adhesion and biofilm development

4.1. Basics of cell adhesion

Settlement of a solid surface by a microbial biofilm is highly dependent on the preliminary attachment of cells to the support. It is agreed that biofilm formation takes place in eight successive steps (Characklis and Marshall, 1990) and the term "cell adhesion" covers a complex mechanism (Berkeley, 1980; Marshall, 1984), which commonly involves the first three steps.

The first step is the formation of a conditioning film on the surface. This is composed of organic or inorganic matter. By modifying the surface charge, potential, and possibly topography, it can promote or lessen bacterial adhesion.

The second step involves a random or targeted contact between planktonic cells and the surface. This step has been approached using theories developed in colloidal science. There, physical forces are at play: if the attractive forces are greater than the repulsive forces, bacteria reversibly adsorb to the surface. The forces to be considered include Van der Waals forces, steric interactions and electrostatic interactions, which are strongly dependent on the compositions of the conditioning film and the medium. Interfacial interactions have been modelled using the DLVO theory applied to cells (Marshall et al., 1971), which has been supplemented by an extended DLVO theory adding hydrophobic/hydrophilic and osmotic interactions (Van Oss et al., 1986; Van Oss, 1989; Hermansson, 1999). These forces act at the nano-scale and are classified as long-range (< 150 nm), such as Van der

Waals and electrostatic double-layer forces, or short-range (< 3 nm) forces, such as hydrogen bonding, ionic and dipole interactions, and hydrophobic forces (Busscher and Weerkamp, 1987). In the case of adsorption of solid particles, the DLVO theory has been complemented with a surface roughness parameter (Czarnecki and Warszyński, 1987).

The application of DLVO theory to the adhesion of microbial cells has been strongly debated. The wall of microbial cells cannot be considered as a well-defined, hard, non-permeable, uniform sheath, as the conventional colloidal approaches assume, but should be approached by soft, permeable heterogeneous interphases with a non-negligible thickness (Gaboriaud et al., 2008; Duval and Gaboriaud, 2010; Hori and Matsumoto, 2010). The complexity of the theoretical model is consequently significantly increased.

The third step of the adhesion process, irreversible adhesion, involves molecular reactions. Bacterial surface structures, such as adhesins and transmembrane polymers, perform chemical bridging with the conditioning film of the surface (Davey and O'toole, 2000; O'Toole et al., 2000; Ubbink and Schaer-Zammaretti, 2007). The bonds must be strong enough to overcome the repulsive and detachment forces, such as shear forces induced by fluid flow. Increasing the contact area between bacterial cells and the electrode surface gives more opportunities for linking and thus increases the adhesion strength. Once they are irreversibly bonded with the surface, bacteria are able to change their metabolism, switching from a free-swimming way of life to a complex surface-attached community life (Flemming et al., 2016).

4.2. Basics of biofilm development and structural models

As the primo-adherent cells undergo irreversible adhesion, they adopt a biofilm metabolism (Costerton et al., 1994; Costerton, 1995), which is expressed by the growth and division of the cells to form microbial aggregates. The growth of such cell clusters and their spatial arrangement create the three-dimensional structure of the biofilm (Klapper and Dockery, 2002). Biofilm growth and maturation involve the establishment of an extracellular matrix that holds the three-dimensional structure of the biofilm. The extracellular matrix ensures the structural integrity of the microbial community, protects it from environmental stresses, and enhances nutrient availability (Cogan and Keener, 2004; Flemming et al., 2007; Flemming and Wingender, 2010).

The formation and growth of a biofilm has been described as a developmental process (Doyle, 2001) evolving from primo-adherent cells to structured microbial aggregates and extracellular matrix (Characklis and Wilderer, 1989) to the final organised microbial biofilm community. Understanding the mechanisms that govern how this complex organization becomes established in connection with the environmental factors has been a great challenge for the microbiology community in the past thirty years (Goller and Romeo, 2008). It has led to detailed understanding of the causal relationships linking genotype to phenotype within the biofilm communities (Monds and O'Toole, 2009), through genomic approaches linked with quorum sensing studies (Nealson et al., 1970; Eberhard, 1972; Irie and Parsek, 2008). In this context, the chemical and topographical features of surfaces have been shown to influence metabolic changes critical for biofilm formation (Shemesh et al., 2010). Nevertheless, there are still very few studies that have addressed the link between the properties of a surface and the response of bacterial communities growing on it.

4.3. Influence of surface topography on cell adhesion and biofilm formation

As detailed above (Section 4.1), cell adhesion mechanisms have mainly been approached by theories developed in the domain of colloidal particles. In this context, surface topography has rarely been identified as an essential parameter that may strongly influence cell adhesion. Actually, DLVO studies are generally carried out by considering particles with a perfectly smooth surface and in the absence of shear stress. Yet shear stresses due to solution flow are predominant in most aqueous

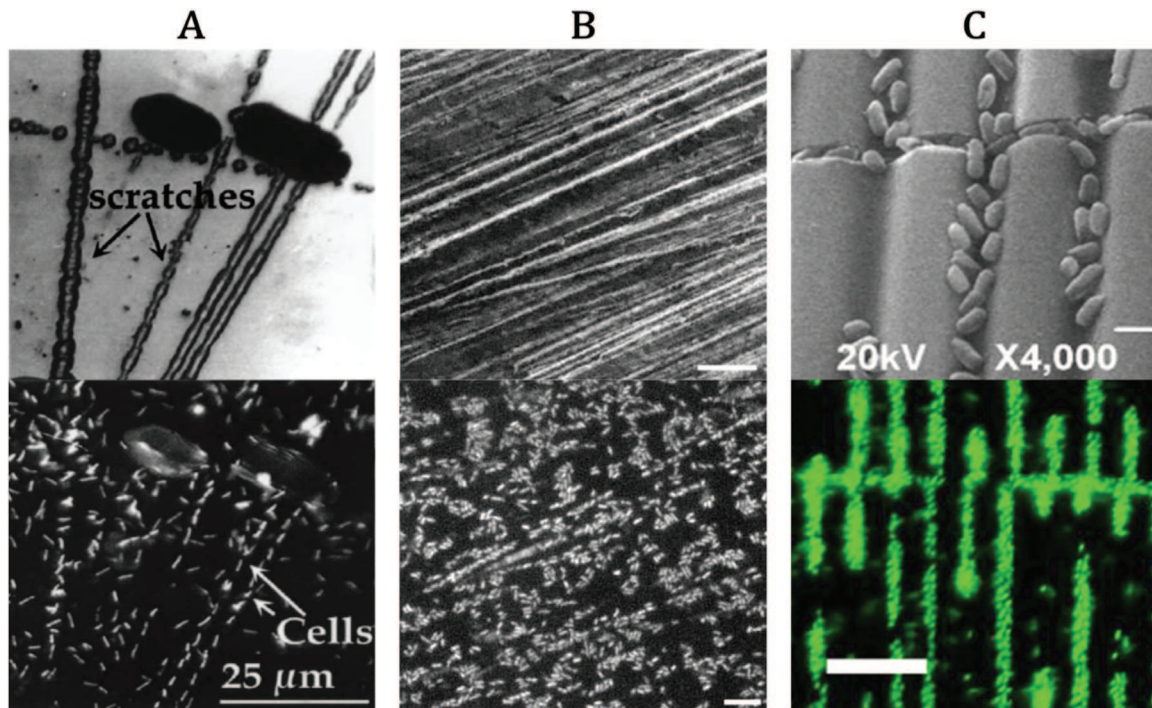


Fig. 2. Preferential adhesion of bacteria in scratches and valleys observed by SEM (top images) and epifluorescence microscopy (bottom images). (A) From [Edwards and Rutenberg \(2001\)](#), cells from a soil enriched culture on a pyrite surface: polishing the surface created micrometric scale asperities that retained and oriented the cells. (B) From [Medilanski et al. \(2002\)](#), *D. desulfuricans* on a stainless steel surface polished with silicon carbide paper: bacterial colonization was concentrated and oriented along the micrometer-scale grooves. (C) From [Epstein et al. \(2013\)](#), attached cells of *P. aeruginosa* colonizing a PDMS surface patterned with wrinkles in a flow cell: the colonization only occurred in the calmer spaces formed by the 4- μm spaced wrinkles; high colonization was also observed on the perpendicular crack where the cells were entrapped.

environments used to grow microbial cells. Neglecting the solution flow has consequently been judged as a possible source of discrepancy between the colloidal-based theoretical approaches and the real world ([Perni et al., 2014](#)).

When shear stress is taken into account, the surface topography takes on great importance since it can create local variations of the shear forces. It has been shown that, on altered surfaces, the initial adhesion of bacteria is favoured by asperities and tends to occur in the low shear force areas like scratches, holes or valleys ([Characklis and Marshall, 1990](#)). These observations have been reported in many studies as illustrated in [Fig. 1](#). They have been confirmed by implementing well-controlled surface topographies obtained by patterning the surface with micro-holes and micro-pillars ([Díaz et al., 2007a, 2007b, 2010; Lorenzetti et al., 2015; Hochbaum and Aizenberg, 2010; Helbig et al., 2016](#)) ([Fig. 2](#)). It now seems established that bacterial settlement on a surface is enhanced by micro-roughness that creates low-shear-force areas and thus provides the cells with calm spaces for adhesion.

Topographies with sizes of the same order of magnitude as those of cells have been claimed to be the most efficient to increase bacterial retention ([Helbig et al., 2016](#)). On the one hand, they present local sites with diminished shear forces and, on the other, they offer a greater contact area to bacteria ([Flint et al., 2000; Edwards and Rutenberg, 2001; Medilanski et al., 2002](#)) ([Fig. 3B](#) and [Fig. 4C](#)). When the surface topographical size increases, shear forces are still reduced but the reachable contact area no longer increases ([Fig. 3B](#) and [Fig. 4D](#)). The surface can be considered as flat at the scale of the microbial cells. In this case, cell adhesion can be enhanced because of the reduction of shear stress but there is no longer the effect due to the increase of the contact area.

At the nano-scale, roughness does not create calm spaces that could be exploited by the cells to adhere and it does not significantly increase the possible contact area. It has even been reported that nano-roughness can reduce the contact area between surface and cells ([Ivanova](#)

[et al., 2011](#)) ([Fig. 4B](#)). Thus nano-roughness and micro-roughness are claimed to have opposite effects on the bacterial adhesion ([Fig. 3C](#)). While nano-roughness tends to reduce the attachment strength, micro-roughness increases it and improves the adhesion environment. This difference has been confirmed by using Atomic Force Microscopy (AFM) to measure the strength of bacterial attachment. On stainless steel that presented different R_a below 0.8 μm ($R_z = 0.3 \mu\text{m}$ and $R_{Sm} = 2.8 \mu\text{m}$), forces of 8 nN were necessary to detach 50% of the cells, whereas surfaces with R_a of around 10 μm ($R_z = 0.04 \mu\text{m}$ and $R_{Sm} = 0.96 \mu\text{m}$) showed 50% of cell detachment at only 4 nN ([Boyd et al., 2002; Whitehead et al., 2006](#)). The strength needed to detach bacteria from the support was twice as high on a surface with topography of the micron scale as on a surface with submicron scale topography.

Nevertheless, nano-roughness has sometimes been claimed to significantly affect cell adhesion through indirect pathways. For instance, nano-roughness has been observed to lower the attachment of proteins to the surface by altering their supramolecular assemblages and thus causing bacterial attachment and retention to dwindle ([Denis et al., 2002](#)). Some studies have even demonstrated that the nano-topography of a surface could inactivate some proteins ([Müller, 2001](#)).

Nano-roughness has also been claimed to increase cell adhesion by increasing the surface hydrophobicity. Surfaces patterned with titanium nano-wires of different diameters exhibited higher roughness as the diameter of the wires decreased ([Anitha et al., 2015](#)). Ranging from R_a of 11 nm ($R_{max} = 20 \text{ nm}$) to R_a of 17 nm ($R_{max} = 35 \text{ nm}$) for wire diameters of 140 and 20 nm, respectively, the 1.5-fold increase of roughness resulted in a 4 fold increase in the attached biomass. This was explained by the higher hydrophobicity of the rougher surfaces (contact angles of 32.4° and 57.6° for R_a of 11 and 17 nm, respectively).

A surface patterned with nano-pores of various diameters from 15 to 100 nm showed that the electrostatic repulsive forces resulting from the nano-pores opposed bacterial adhesion. The electrostatic repulsion was combined with acid-base repulsion and impacted all types of the tested

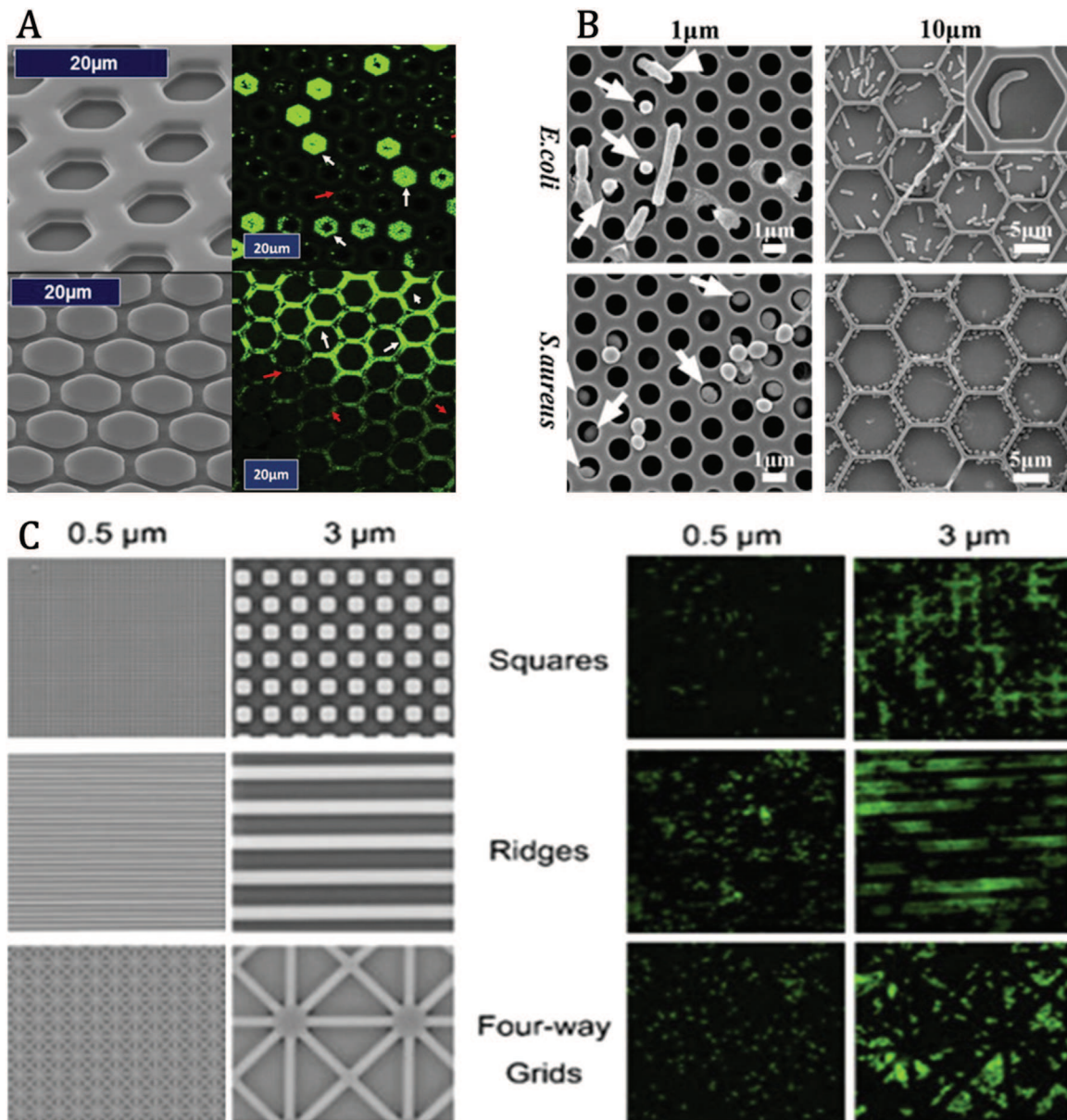


Fig. 3. SEM and Epifluorescence microscopy of various patterned surfaces and their microbial colonization. (A) From Vasudevan et al. (2014), *E. cloacae* was cultured on two PDMS surfaces patterned with hexagonal pits and pillars; the cells colonized only the recessed regions inside the pits or spaces between the pillars, which are protected areas. (B) From Yang et al. (2015), rod-shape bacteria *E. coli* and spherical-shaped bacteria *S. aureus* were inoculated on silicon surfaces presenting 1- μm holes and 10- μm hexagonal pits; white arrows show entrapped *S. aureus* cells (1 μm spheres) and *E. coli* cells (3–4 μm rods) only partially entrapped in the 1- μm holes; this pattern is highly efficient in terms of retention for bacteria having sizes similar to those of the holes; on the 10 μm pits, the reduction of shear forces was less and, consequently the *S. aureus* cells were only retained on the wall side, where they had a higher contact area with the surface. In contrast, elongated *E. coli* cells presented a higher contact area with the surface, allowing them to adhere even far from the pit walls. (C) From Lu et al. (2016); on patterned PDMS surfaces inoculated with *P. aeruginosa*, very little bacterial adhesion was observed when the patterning dimension was under 1 μm because the cells could not penetrate into the patterned surface; they could not take advantage of the low shear forces and had a smaller contact area with the surface (see Fig. 3.B).

bacteria (Feng et al., 2015). It has been reported that the impact of nano-roughness is dependent on the cell type and shape (Ivanova et al., 2011). Surfaces with 0.18 and 0.52 nm R_a and -0.08 and 0.43 R_{sk} showed different colonization patterns of rod-shaped and spherical bacteria.

Nano-roughness has also been claimed to affect the phases of bio-film development that occur after the adhesion of individual cells. It has been reported to induce changes in the extracellular polymeric matrix and even in the ratio between the proteins, polysaccharides and lipids that compose the matrix (Truong et al., 2009; Singh et al., 2011) and the amount of matrix with respect to cell density (Singh et al., 2011). On a nano-rough surface, cells are in contact with only a few peaks

(Fig. 4B), which may result in weak attachment to the surface. In such a situation, cells have been assumed to compensate for the lack of cell/surface contact points by secreting a matrix that is more efficient at gluing them to the surface.

5. Impact of electrode topography on microbial electrochemical systems

5.1. General overview

Surface roughness is not absolutely necessary to form a microbial electrode as it has been reported that an ultra-flat gold electrode can

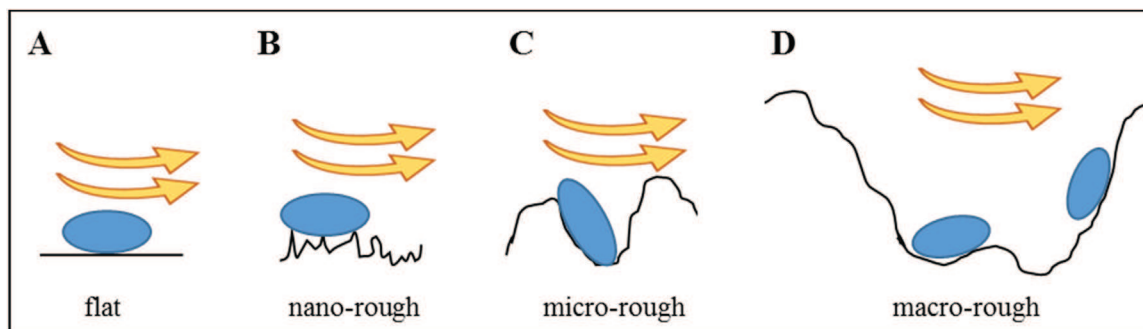


Fig. 4. Impact of contact surface and shear forces on bacterial adhesion depending on the surface roughness scale. On the flat and nano-rough surfaces (A, B), the cell is directly impacted by the hydrodynamic flow and is subjected to high shear forces. On micro- and macro-rough surfaces (C, D), the hydrodynamic flow is reduced in the grooves of the surface structure, which provides the cell with a calmer space to adhere. Regarding the contact area between the cell and the surface, the binding surface is reduced in the nano-rough configuration (B) and enhanced when the roughness is of the same order of magnitude as the cell dimensions (C).

hold an electroactive biofilm that is as efficient as one developed on a rough carbon cloth electrode (Richter et al., 2008). Nevertheless, it is generally accepted as a rule of thumb that increasing the electrode surface roughness increases the electrochemical performance of microbial electrodes (Dumas et al., 2008b; Erable et al., 2009b, 2010; In Ho et al., 2011; Cercado-Quezada et al., 2011; Ye et al., 2012; Bombelli et al., 2012; He et al., 2012; Kim et al., 2014; You et al., 2014; Guo et al., 2014; Thung et al., 2016; Tao et al., 2016). Some studies comparing different electrode materials have even suggested that the surface roughness may have a greater impact than the nature of the material itself (Dumas et al., 2008b; Erable et al., 2009b). The better performance observed with the rougher electrodes has most often been attributed to enhancement of cell adhesion and biofilm development (Erable et al., 2009b, 2010; In Ho et al., 2011; Ye et al., 2012; You et al., 2014; W. Guo et al., 2014; Thung et al., 2016). The fact that biofilm development is favoured by the surface roughness has even been identified as a possible disadvantage for long-term operation because biofilm overgrowth may finally result in a decrease in long-term performance (Thung et al., 2016). In contrast, some studies have reported that the rougher electrodes produce higher current, even though no significant difference in biofilm development was observed (Dumas et al., 2008b; Bombelli et al., 2012).

Numerous articles have noted the effect of surface roughness on the current produced by microbial electrodes or on the power produced by microbial fuel cells, although it was not the main purpose of the study. In this context, several basic explanations have been suggested, sometimes only as speculative ideas that should be investigated further. The increase of surface area available for cell adhesion has been evoked to explain greater biofilm development (You et al., 2014). In contrast, the importance of the larger available area has sometimes been denied by pointing out the more significant effect of the difference in surface energy linked to different roughnesses (Bombelli et al., 2012). The lower charge-transfer resistance of rougher electrodes (Ye et al., 2012) and a difference in local acidification of the biofilm (Kim et al., 2014) (see Section 5.3) have also been evoked.

Actually, the main aim of most of these studies was to improve the current produced by the electrode or the power supplied by the microbial fuel cell, so the accurate characterization of the effect of surface topography was not an essential objective in this context. Drastic procedures were consequently used to modify the electrode surface, such as plasma (He et al., 2012), electrochemical oxidation (Cercado-Quezada et al., 2011) and various chemical surface modifications (Lai et al., 2011; Bombelli et al., 2012; You et al., 2014; Guo et al., 2014), and some studies compared electrodes of different materials. In such conditions, the differences in surface roughness were only a consequence of the modification of other physicochemical parameters that may also play a key role in cell adhesion, biofilm formation and electron transfer rate, such as hydrophilicity (Zhou et al., 2017), surface

energy (Bombelli et al., 2012) and surface chemical composition (Cercado-Quezada et al., 2011; Lai et al., 2011). In this context, the effect of surface roughness could not be differentiated from the effect of the other parameters that were changed.

From this preliminary overview, it can easily be concluded that surface roughness most probably has an impact on microbial electrodes and that this issue would deserve specifically dedicated studies. These studies should be designed to minimize the number of parameters that are modified when modifying the surface roughness. The detailed review presented above is based on the articles that have tended towards this objective.

5.2. Random micro-roughness (Table 2)

The limit between nano-, micro- and macro-roughness is still unclear and may vary with the surface under study. Thus it is difficult to clearly attribute an effect to each of the roughness scales but recent works have been focusing on this issue (Santoro et al., 2014; Li et al., 2014). The first study segregated roughness in two ranges: from 5 to 10 μm , where roughness was characterized by the high frequency roughness R_a^H and skewness R_{sk}^H , and from 100 to 300 μm , characterized by low frequency roughness R_a^L and skewness R_{sk}^L (Santoro et al., 2014). This classification could be related to micro- and macro-roughness. Various commercial carbon papers implemented with wastewater in a microbial fuel cell showed that the decrease of bacterial attachment and current density was correlated with the decrease of R_{sk}^H , while no correlation could be established with the variation of R_{sk}^L . This means that the topographical variations measured at the scale of 5–10 μm , close to the cell size, had an impact on the system while the variations in the 100–300 scale were irrelevant in an analysis of the microbial fuel cell performance. In order to better differentiate these impacts, a segregation into four ranges was studied (20–100 nm, 0.9–5 μm , 6.5–50 μm and 163–450 μm) but it was not possible to correlate the different parameters with the bacterial adhesion or with the current produced. This study illustrated how complex surface characterization can be (Li et al., 2014).

As recalled in Section 4.3, when approaching the size of a cell, around a few micrometers, roughness offers an obvious physical benefit for bacterial adhesion by creating “calm areas” and increasing the reachable contact area. The results presented above tend to confirm this and the phenomenon, well-documented in the field of biofilms in general, seems to also apply to electroactive biofilms. It has also been observed on stainless steel electrodes inoculated with *Geobacter sulfurreducens*. Increasing the roughness from 0.1 to 2 μm produced almost no effect on biofilm coverage and electrical output, while a shift from 2 to 4 μm multiplied the current density provided by the cathode by 1.6 (Pons et al., 2011b). The current rise was explained by the presence of dense bacterial colonies on the roughest, 4 μm , surface, which formed

Table 2
Performance of microbial anodes with random micro-rough surface.

References	Testing device	Bacteria	Substrate	Anode materials	Surface characteristics	Maximal density	
						Current (A m ⁻²)	Power (W m ⁻²)
Santoro et al. (2014)	MFC	Wastewater	Sodium acetate (1 g L ⁻¹)	Carbon paper with various PTFE content	0 wt% PTFE :	0.13	
					Ra = 52 μm	0.19	
Li et al. (2014)	MFC	Wastewater	Sodium acetate (1.5 g L ⁻²)	Raw carbon papers and electrochemically oxidized with three electrolyte	60 wt% PTFE:	1.5	
					Ra = 136 μm	0.16	
					No treatment :	1.7	
					Ra = 15.5 μm	0.2	
					HNO ₃ / H ₂ SO ₄ :	1.75	
Pons et al., 2010	Three-electrode set-up. Electrode polarised at - 0.5 V vs. Ag/AgCl	<i>Geobacter sulfurreducens</i>	Sodium acetate (0.82 g L ⁻¹)	Sandblasted stainless steel	Ra = 16.5 μm	0.18	
					NH ₄ NO ₃ :	0.6	
					Ra = 13.5 μm	0.6	
					(NH ₄) ₂ SO ₄ :	1	
					Ra = 13.8 μm	1	
Pocaznoi et al. (2012a)	Three-electrode set-up. Electrode polarised at - 0.2 V vs. SCE	Leachate from garden compost	Sodium acetate (1.64 g L ⁻¹)	Sandblasted stainless steel	Ra = 0.1 μm	19.7	
					Ra = 2.3 μm	21.5	
					Ra = 4 μm		
					Ra = 6.1 μm		
					Ra < 0.9 μm		

in the calm spots created by the valleys.

In contrast, another study performed on stainless steel with a multi-species bacterial inoculum showed no noticeable effect of either the large (300 μm wide, 500 μm deep channels) or the small values of micro-roughness (R_a = 5 μm) compared with those for smooth stainless steel (Pocaznoi et al., 2012a). The effect of micro-roughness seems to be subject to strong variation depending on the system and the bacterial inoculum, and it may be higher in restricted growth conditions.

It has also been observed that the shape and the size of the microbial colonies induced by the micro-roughness may affect their electrochemical properties. In the case of *G. sulfurreducens* colonies settling on rough stainless steel electrodes, the current density calculated with respect to the surface area covered by the bacterial colonies, instead of the whole electrode surface area, was shown to decrease with bacterial coverage (Pons et al., 2011a). Isolated cells and small colonies provided higher local current density than dense colonies. The total current increased with the global biofilm coverage ratio but, locally, small colonies proved more efficient than big microbial aggregates. This may open up valuable possibilities for micro-scale surface engineering of electroactive biofilms.

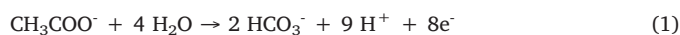
5.3. Surface micro-structuring (Table 3)

Studies of the impact of the electrode topography should gain considerably in accuracy when well-defined electrode surface structuration is implemented. Electrode surfaces with regular topography can thus be offered to bacterial settlement in a fully reproducible manner.

The pioneering works in this field were conducted by the MEMS research community, which previously used electrode structuration for biological applications (Grayson et al., 2004). In the context of MFC, micro-structured gold electrodes presenting arrays of 8-μm-high cross-shaped micro-pillars of 40 μm width, inoculated with *Saccharomyces cerevisiae*, produced a current density of 30.2 μA cm⁻² (Siu and Chiao, 2008). This current density was 4.9 times that obtained on flat gold although the surface area was only increased 1.8 times. It is stated that micro-pillars reduced the culture time of the MFC by a factor of 4 and thus increased the current conversion of substrate.

Another study performed with a *Serratia marcescens*-inoculated MFC, which implemented micro-structured anodes with cylindrical micro-pillars of 20 μm diameter, 20 μm height and various spacings from 20 to 50 μm, highlighted the impact of micro-structuring on a key

issue for electroactive biofilm (Kim et al., 2014). It showed that the micro-pillar pattern reduced the local acidification of the biofilm and thus led to a lower rate of microbial death. Actually, the oxidation of organic compounds, e.g., acetate, which is the most usual substrate for microbial anodes, leads to the production of protons:



and electroactive biofilms are very sensitive to low pH. Local acidification has been demonstrated to be a major cause of inhibition of microbial anodes (Torres et al., 2008; Popat and Torres, 2016; Oliot et al., 2016)

The protective effect of micro-pillars against local acidification was accentuated by the densification of pillars. A 14% improvement of the power density was reported with the tightest micro-pillar pattern in comparison with the smooth surface, thus reaching 287 μW cm⁻² which corresponds to 1.3 μA cm⁻² current density. The biofilm developed on the 20-μm spaced pillars was thinner than on the 50-μm ones. It was concluded that the biofilm acidification caused a thickening of the biofilm due to a high death ratio. In contrast, in the presence of structuration, biofilm formation was delayed and proton accumulation and the death ratio were thus reduced. Micro-pillars provided an appropriate environment for the formation of a thin and effective electroactive biofilm by mitigating the acidification phenomenon.

A recent study using gold micro-pillars of 500 μm height has come to a seemingly opposite conclusion (Champigneux et al., 2018). Firstly, it was observed that the presence of micro-pillars favoured reproducible full biofilm coverage, and increased the current density to 8.5 A m⁻², while the flat surface gave only 2.5 A m⁻². This increase was due only to the increase of the surface area that was available for microbial settlement. In contrast to the study described above, numerical modelling explained that the micro-pillar structure increased the local acidification by hindering the transfer of buffering species inside the micro-pillar array. Actually, these apparently opposite conclusions were obtained in different operating conditions: micro-pillars of 20 μm vs. 500 μm, mature biofilms vs. early colonization, MFC vs. electro-analytical set-up with control potential, and different strains, which led to different current densities.

Ultra-microelectrodes (Heinze, 1993; Bard and Faulkner, 2001) have been implemented in the form of gold micro-lines of 10 μm width spaced by 100 μm of non-conductive material (Liu et al., 2010) and inoculated with *Geobacter sulfurreducens*. The hemispheric biofilms that developed over the gold micro-lines resulted in a larger amount of

Table 3
Performance of microbial anodes with micro-structured surface.

References	Testing device	Bacteria	Substrate	Anode surfaces	Surface characteristics	Maximal density	
						Current (A m ⁻²)	Power (W m ⁻²)
Siu and Chiao. (2008)	MFC connected to resistance (47–1 kΩ)	<i>Saccharomyces cerevisiae</i>	Glucose (180 g L ⁻¹)	Gold deposit structured on PDMS (0,2 μm thick)	Flat surface	6 . 10 ⁻³ (100 Ω)	1 . 10 ⁻⁴
					Around 70 000 pillars 8 μm high, 40 μm wide	3 . 10 ⁻² (100 Ω)	4 . 10 ⁻³
Kim et al. (2014)	MFC	Flagellated gram negative bacteria, <i>Serratia marcescens</i>	Glucose (25 g L ⁻¹)	Cr/Au evaporated (30/300 nm) Round micropillars (20 μm diameter and 20 μm height) Various spacing	No pillars	1.5	2.5
					50 μm spaced pillars	1.7	2.74
					30 μm spaced pillars	1.75	2.68
					20 μm spaced pillars	1.82	2.87
Champigneux et al. (2018)	Three-electrode set-up. Electrode polarised at + 0.1 V vs SCE	<i>Geobacter sulfurreducens</i>	Sodium acetate (0.82 g L ⁻¹)	Electrodeposited gold Square micropillars (100 × 100 μm, height 500 μm) Various spacing	No pillars	2.5	
					200 μm spaced pillars	6.1	
					125 μm spaced pillars	7.85	
					100 μm spaced pillars	8.45	
Liu et al. (2010)	Three-electrode set-up. Electrode polarised at + 0.24 V vs SHE	<i>Geobacter sulfurreducens</i>	Sodium acetate (2.46 g L ⁻¹)	Gold deposit on silicon wafer (0.75 μm thick)	Flat and plain gold	4	
					10 gold lines of 1,5 cm × 10 μm spaced by 100 μm	16	
Pocaznoi et al. (2012b)	Three-electrode set-up. Electrode polarised at - 0.2 V vs SCE	Mixed bacterial community from compost leakages	Sodium acetate (0.82 g L ⁻¹)	Platinum wire of various diameter	500 μm Ø	8	
					50 μm Ø	20	
Ren et al. (2016)	MFC connected to resistance (148–1 MΩ)	<i>Geobacter</i> -enriched community from anaerobic-digestion sludge	Sodium acetate (2.05 g L ⁻¹)	Gold deposit on glass (0.42 μm thick) Imbricated 20 μm wide rings electrodes with various gaps.	25 μm Ø	66	
					Flat and plain gold	5.2	2.1
					100 μm spaced rings	17.7	6.9
Inoue et al. (2012)	MFC connected to resistance (lowered stepwise)	<i>Geobacter sulfurreducens</i>	Sodium acetate (0.82 g L ⁻¹)	Au/Cr deposit on Si (400/200 nm thick)	220 μm spaced rings	15	7.7
					Pattern of 5 μm x 5 μm holes	6 . 10 ⁻³	
Kano et al. (2012)	MFC connected to resistance (100–3000 Ω)	<i>Shewanella putrefaciens</i>	Glucose (0.4 g L ⁻¹)	Au evaporated Patterned with 1 μm deep square holes	Pattern of 5 μm wide / 3.6 μm deep channels	2 . 10 ⁻³	
					1 μm wide		0.18
					2 μm wide		0.27
					3 μm wide		0.3
					4 μm wide		0.38
					5 μm wide		0.41
7 μm wide		0.38					
10 μm wide		0.35					

bacterial biomass related to the electrode surface area in comparison to biofilms developed on plain macro-electrodes. The largest amount of biomass linked to a micro-line electrode resulted in 4-fold higher current density (1.6 mA cm⁻²) than on a plain gold surface (0.4 mA cm⁻²). A limitation due to extracellular electron transfer was assumed when a critical thickness of 20 μm was reached.

Likewise, biofilms formed from a multi-species inoculum around a cylindrical ultra-microelectrode of 25 μm diameter produced up to 6.6 mA cm⁻² (Pocaznoi et al., 2012b). In this case, it was assumed that the extracellular electron transport network was improved by the ultra-microelectrode effect. Same hypothesis was discussed with ring electrodes 20 μm wide and spaced by 100 and 220 μm of uncondutive material (Ren et al., 2016). A 50 μm hemispherical biofilm developed on the ultra-microelectrodes resulting in 3-fold increase with current density up to 17.7 A m⁻².

Micro-structuring has also been achieved with micro-holes (5 μm wide and 8 μm deep) and micro-channels (5 μm wide and 3.6 μm deep) (Inoue et al., 2012). When they were inoculated with *Geobacter sulfurreducens*, a good correlation between the surface area and the current production was reported but no supplementary positive or negative effect of the micro-structuration was observed.

The effect of the size of micro-holes has been investigated with gold electrodes having square holes with sides ranging from 1 to 10 μm, inoculated with *S. putrefaciens* (size 3–5 μm) (Kano et al., 2012). Implemented in an MFC, the microbial anodes with holes of 4 and 5 μm sides led to the highest power. The optimal size of the micro-holes corresponded to the size of the bacteria. It was concluded that offering an increased contact area to the cells resulted in more efficient electron

transfer, which was consistent with the direct electron transfer pathway achieved by *S. putrefaciens* (Kim et al., 1999) (see Section 4.3). However, it may be noted that micro-holes of side 4–5 μm can increase the contact area for individual bacteria but they may be less favourable for biofilm development than micro-pillar structures because they isolate cells or colonies from each other (Vasudevan et al., 2014; Yang et al., 2015).

5.4. Nano-roughness (Table 4)

At the nano-scale, it is difficult to make a strict distinction between random nano-roughness, which is generally obtained by global surface treatments, and nano-structuring, which should result in well-patterned surfaces. For instance, it is hard to decide whether surface modification with nanoparticles, nanowires or nanotubes belongs to one or other of the categories. We have consequently tried to order this section from random nano-roughness to perfectly patterned nano-structures without drawing a frontier between the two categories.

Glassy carbon electrodes inoculated with *Shewanella oneidensis* displayed a well-developed biofilm on the roughest surface (R_a = 86 nm, R_q 116 nm), whereas the smoothest one (R_a = 11 nm, R_q = 14 nm) was only settled by isolated cells (Ye et al., 2012). In addition, as observed in abiotic systems (see Section 3.2), the nano-roughness also affected the polarization resistance, rougher surfaces showing lower polarization resistance (850 against 12500 Ω). A similar coupled effect of different phenomena has been reported with anodes fabricated by ion implantation of N⁺ on a carbon paper surface. Implantation of N⁺ resulted in roughness being increased to around 100 nm and also

Table 4
Performance of microbial anodes with nano-rough surface.

References	Testing device	Bacteria	Substrate	Anode surfaces	Surface characteristics	Maximal density	
						Current ($A m^{-2}$)	Power ($W m^{-2}$)
He et al. (2012)	MFC	Acclimated anaerobic sludge from anaerobic reactor	Sodium acetate ($1 g L^{-1}$)	Carbon paper implanted with N^+ ions	No implantation Implanted N^+ ions : roughened surface		0.033 0.1
Guo et al. (2014)	Three-electrode set-up. Electrode polarised at $-0.2 V$ vs. $Ag/AgCl$	Anodic effluent of an existing BES, which is rich in <i>Geobacter</i>	Sodium acetate ($2 g L^{-1}$)	Stainless steel felt flame oxidized	Untreated : $R_a = 75 nm$ Oxidized: $R_a = 151 nm$	1.1 19.2	
Alatraktchi et al. (2012)	DCMFC	Anodic effluent of a two-chamber MFC with modified domestic wastewater	Sodium acetate ($1.33 g L^{-1}$)	Gold Carbon paper	Plain gold Nanograss 100s of nm high Untreated carbon paper Gold nano-particules sputtered carbon paper 10s nm high		$3.9 \cdot 10^{-5}$ $2.5 \cdot 10^{-3}$ 0.11 0.35
Jia et al. (2016)	MFC connected to resistance (80Ω to $40 k\Omega$)	<i>Shewanella loihica</i>	Sodium acetate ($0.82 g L^{-1}$)	Carbon paper with TiO_2 nanowires	Untreated carbon paper Fibers covered with 10 nm wide titanium wires mattress		0.19 0.39
Feng et al. (2016)	MFC connected to resistance (1Ω)	Anodic effluent of an acetate-fed MFC reactor	Sodium acetate ($1 g L^{-1}$)	Titanium plate with TiO_2 nanotubes	Bare titanium nanotubes diameter of 60-90 nm, Roughness around 10 s of nm	0.066 12.7	
Champigneux et al. (2018)	Three-electrode set-up. Electrode polarised at $0.1 V$ vs. SCE	<i>Geobacter sulfurreducens</i>	Sodium acetate ($0.82 g L^{-1}$)	Gold	Smooth: $R_a = 0.8 nm$ Rough : $R_a = 4.5 nm$	0.9 2.5	
Ye et al. (2017)	Three-electrode set-up. Electrode polarised at $0.24 V$ vs. SHE	<i>Shewanella oneidensis</i>	Lactate ($1.8 g L^{-1}$)	Glassy carbon plates	Unstructured glassy carbon Grid patterned at 115 nm high Grid patterned at 300 nm high	0.12 0.25 0.12	
Ye et al. (2012)	Three- electrode set-up	<i>Shewanella oneidensis</i>		Glassy carbon plates	$R_a = 11 \pm 0.5 nm$ $R_a = 88.6 \pm 13.4 nm$	Electrode resistance :	12 500 Ω 850 Ω

decreased the resistance and increased hydrophobicity (He et al., 2012). It was consequently not possible to discriminate between the contributions of the three phenomena to the 3-fold power density increase obtained by implementing the electrodes in an MFC inoculated with sludge.

At the nano-scale, did the roughest surface enhance the development of electroactive biofilms by favouring bacterial adhesion and biofilm formation or by decreasing the polarization resistance, i.e. improving electron transfer? Bacteria use electron transfer to the electrode to support their metabolic process. Facilitating electron transfer can consequently lead to more intense biofilm development. Did nano-roughness impact cell adhesion directly or did it favour the growth of electroactive bacteria by accelerating electron transfer? A clear causal chain can hardly be extracted yet.

The difficulty of drawing straightforward conclusions has been illustrated by another study performed on stainless steel electrodes that were either treated by flame oxidation or left untreated (Guo et al., 2014). Flame oxidation not only increased the nano-scale roughness from 75 to 151 nm but also altered the surface chemistry. The treatment resulted in better biofilm coverage and higher current output from enriched *Geobacter psychrophilus* and *Geobacter sulfurreducens* inocula for the rougher oxidized electrode but, once again, the improvement cannot be fully related to the increase in surface nano-roughness since the flame oxidation also deteriorated the chromium-rich passive layer. Actually, the nature of the passive layer can significantly affect electron transfer rate with biofilms (Pons et al., 2011a). Similarly, the generation of current by *Geobacter sulfurreducens* has been shown to be influenced by the crystallographic nature and orientation of the electrode surface (Maestro et al., 2014) and the crystallographic state can directly influence the nano-roughness of the electrode surface.

The technique used to achieve the surface nano-topography may also have a considerable impact on the performance of an electroactive biofilm. Grass-like nano-roughness of 100 nm obtained by deep reactive ion etching of a gold electrode has been compared with sputtering of gold nanoparticles on carbon paper in an MFC inoculated with wastewater (Alatraktchi et al., 2012). Nanoparticle sputtering led to considerably higher power densities (346 vs. 2.5 mW/m²). It has generally been observed that using nanoparticles sputtered on smooth electrodes to increase their roughness leads to increased current output (Qiao et al., 2007; Sharma et al., 2008; Sun et al., 2010; Fan et al., 2011; Quan et al., 2015; Z. Lu et al., 2015; Xu et al., 2018) but, since nanoparticle sputtering also impacts the surface chemistry and its electrical characteristics, the current enhancement cannot be linked directly with the nano-topography.

Surfaces modified with titanium oxide nanowires (Jia et al., 2016) and titanium oxide nanotubes (Feng et al., 2016) have shown that a titanium surface with a nanometre organization constitutes a suitable electrode material for forming microbial anodes, in contrast to smooth titanium, which has proved to be of no interest. After inoculation with effluent from an existing acetate-fed MFC reactor, no biofilm developed on smooth titanium, and consequently no current was produced, while the titanium electrodes coated with nanotubes were completely covered with biofilm and produced 12.7 A m⁻². Titanium nanowires, on the other hand, were believed to act as a substitute for bacterial pili by promoting the bacterial adhesion and also favouring electron transfer. Here again, the conclusions should be moderated by considering the low conductivity of titanium oxide. In the field of electrochemical engineering, pure titanium is not considered as appropriate anode material because of the formation of a low-conductive oxide on its surface. Surface modification by titanium nano-objects such as nanowires or nanotubes may mainly act by modifying the surface conductivity.

Very recent studies have tended to single out the effect of nano-topography by comparing surfaces of identical nature. Gold surfaces with R_a of 0.8 and 4.5 nm have been compared. The 4.5-nm roughness was obtained by electrolytic deposition of gold on the 0.8-nm smooth

gold surface (Champigneux et al., 2018). Inoculated with *G. sulfurreducens*, the 0.8 and 4.5 nm-rough electrodes respectively produced 0.9 and 2.5 A m⁻² on average. It was speculated that nano-roughness might act by increasing the electron transfer rate. Another study implemented nano-patterning to design well-controlled grids 3 μm wide and 115 or 300 nm high on glassy carbon surfaces. These electrodes were inoculated with *S. oneidensis* (Ye et al., 2017). The 115 nm high pattern was 78% more effective in terms of cell attachment density than the smooth surface and 40% more productive in current density. The 300 nm high pattern showed no significant improvement in terms of current density compared to the smooth surface, even though the bacterial attachment was a little greater. It was assumed that the 300 nm pattern created frontiers between the biofilm patches that formed on different areas of the grid, and was thus detrimental to global biofilm organization. In contrast, the 115 nm pattern was not too high and did not hinder biofilm unity. The thermodynamic explanation given was that the membrane deformation needed to overcome the 300 nm feature was too demanding in energy. It highlights an optimal feature threshold of around 100 nm, which is consistent with cell energy limitations.

6. Open questions and thoughts

As a first obvious conclusion, according to the impressive influence of the electrode surface topography on microbial electrochemical systems which have already been reported, it can be stated that surface topographical engineering is a very promising avenue for improving the efficiency of electroactive biofilms.

6.1. Take care when choosing the experimental set-up

Two different approaches can be followed to investigate microbial electrochemical systems depending on the objective. The engineering approach implements whole reactors and processes in order to shift the technology towards actual application as fast as possible (Pocaznoi et al., 2012c). In contrast, fundamental investigations try to single out a given phenomenon in order to gain deeper fundamental understanding of it. It is essential not to confuse the two approaches, which correspond to different purposes and different experimental set-ups.

In the context of engineering, with the objective of improving the performance of microbial electrodes, optimal surface topography can be associated with porous electrodes, 3-dimensional structures and surface functionalization, resulting in very complex electrode architecture. This can be illustrated by a glassy carbon electrode coated with carbon nanotubes and then functionalized with polypyrrole and manganese (Lu et al., 2013) or similarly, by carbon material doped with nickel nano-particles, patterned in a micropillar array to form an electrode and then coated with carbon nanofibers (Khare et al., 2016). Such innovative anode designs can lead to valuable improvements in performance but they are not ideally suited to analytical studies that aim to characterize the effects of surface topography on electroactive biofilms. Likewise, an MFC is not an appropriate electro-analytical device (Rimboud et al., 2014), because many other rate-limiting steps than the studied electrode can affect the global performance. As an illustration of this general consideration, it has recently been reported that the cathode roughness can increase MFC performance by improving the electrical contact between the cathode and the current collector (Santoro et al., 2015). In this case roughness had nothing to do with the bioelectrochemical processes. The design of MFCs should be reserved for engineering purposes. A search for a fundamental explanation of the impact of surface topography on electroactive biofilms should be carried out in carefully designed electroanalytical set-ups (Rimboud et al., 2014) with well-defined electrode surface topographies.

6.2. Consensus on the impact of micro-roughness at cell size

Several reports tend to confirm that micro-roughness improves the electroactive biofilm performance in two ways: by offering bacteria sites with low shear forces, suitable for their attachment, and by increasing the bacteria/electrode contact area for stronger attachment and improved electron transfer. These two effects have already been observed with bacterial adhesion and biofilm formation of non-electroactive species (Figs. 1, 2). They are most marked when the average roughness is of the same order of magnitude as the size of the cells, i.e., a few micrometers.

Above a few micrometers, random roughness does not seem to have any great impact on electroactive biofilms, while ordered roughness, which is achieved by surface micro-structuring, may increase the current produced by increasing the surface area available for biofilm development. For microbial anodes, the optimal order of magnitude for micro-structures should be closely related with the diffusion limitation, mainly to mitigate local acidification of the biofilm.

Actually, the impact of uncontrolled random surface topography on electroactive biofilms is still unclear (see Section 5.2), certainly because of the large variety of systems that have been considered in the context of microbial electrochemical systems. Moreover, a large number of these studies have been carried out in MFCs designed for purposes other than characterizing the effect of surface topography. This is another major reason of the lack of clear conclusion. The variation of roughness has most often been the result of physical or chemical surface modifications, making it difficult to discriminate which parameter had the main impact. Specific studies should now focus on roughness as the single parameter tested, taking care to keep the other interfacial properties unchanged. This direction has already been engaged with surface micro-structured electrodes and should be intensified to grasp the real effect of random roughness.

6.3. Interesting possibilities related to nano-roughness (see Section 5.4)

It has been claimed that bacterial cells could hardly grow over a structure 300 nm high due to the deformation limitation. Sharp edges above 100 nm or so may thus delay the formation of continuous biofilms. This gives valuable guidelines on how to favour the development of biofilms on an electrode: nano-structuration may be more efficient when lower than 300 nm or presenting reduced sharpness (R_{ku} lower than 3) and wide peak to peak distance. Conversely, the same guidelines can also be considered if the objective is to hamper the development of harmful biofilm, in the context of biocorrosion for instance.

It has been suggested that bacterial appendices could be mimicked by decorating the electrode surface with metal nano-wires to provide the cells with multiple “pilus-like” nanostructures to achieve extracellular electron transfer. This particular patterning technique may show great promise, but further studies need to be carried out, in particular by avoiding the comparison with flat titanium, which is not an appropriate material for anodes.

At the nano-scale it seems difficult to discriminate between the real effect of the surface topography and that of other interfacial parameters, which are unavoidably changed when nano-topography is modified. Are the characteristics and performance of electroactive biofilms affected directly by the electrode nano-topography or by the impact of the electrode nano-topography on surface chemistry, interfacial resistance, crystallographic state, etc.? (See Section 3.2.) Nano-roughness corresponds to a scale at which it is difficult to avoid other physicochemical properties being affected, so the causal chain can hardly be unravelled. Basic conclusions should be carefully qualified.

6.4. Exciting horizons to be explored

Numerous electrochemical studies have approached, both experimentally and theoretically (see Section 3), the impact of the electrode

roughness on electron transfer in abiotic conditions. This solid basic knowledge should now be exploited for electroactive biofilms. For example, surface nano-roughness has been shown to drastically change the electrochemistry of an equine cytochrome c (Leopold and Bowden, 2002) by acting on the organization of the monolayer that self-assembles on the electrode surface. This type of work, applied to the component of the specific cytochromes c, quinone compounds and other mediator types involved in biofilm extracellular electron transfer (Lidan et al., 2014), could lead to a more accurate description of certain transfer mechanisms.

Fascinating thoughts have been voiced on the possible impact of nano-roughness from the standpoint of the metabolic changes it may induce for adhering bacteria. A microbiological approach through genomic analysis should be suitable to better understand bacterial response to the surface topography. For example, effects of roughness on extracellular matrix production and composition have already been evoked (see Section 4.3) but no experimental confirmation has yet been given. Genomics could be of great help in unravelling the bacterial mechanism at play.

This issue should have great importance for electroactive biofilms because the extracellular matrix plays a crucial role both in biofilm structure and extracellular electron transfer. Using the electrode topography to act on biofilm properties, such as thickness, structure and, above all, conductivity, should be a great breakthrough for electroactive biofilm design. In this direction, in the case of multispecies inocula, it seems very attractive to investigate the possibility of orienting the selection of the suitable electroactive species by means of the electrode topography. For both pure cultures and multispecies inocula, it has been shown that multiple pathways could be involved in extracellular electron transfer (Zhu et al., 2012; Rimboud et al., 2016). Trying to orient the synthesis of the most suitable extracellular mediator(s) through the nano-topography of the electrode would be a very attractive way to optimize electroactive biofilms. This research direction seems very ambitious but it is supported by previous studies. These studies did not deal with electroactive biofilms but suggested that the production by bacterial cells of compounds involved in biofilm structuring may be affected by the nano-topography of the support (see Section 4.3).

7. Conclusion

Surface topography has an obvious, strong impact on the formation and performance of electroactive biofilms. At the scale of around a hundred micrometers and above, surface engineering can be used to increase the electrode surface area. Optimal topographies are likely to be found by improving mass transfer and mitigating local acidification inside the micro-structure. At the micrometer scales, local shear stress and cell-electrode contact angle seem to play the major roles. The nano-scale opens up thrilling horizons, with some hope of acting on the intimate mechanisms of biofilm formation and extracellular electron transfer.

Up to now, many studies have investigated surface topography in parallel with other surface modifications, thus leading to a lack of specific understanding. Conversely, it may be questioned whether the improvements sometimes attributed to chemical or physicochemical modifications of the surface were not, to some extent or even totally, due to modification of the surface topography. The main conclusion of this review is that it may be advisable to develop investigations of well-controlled roughness by taking care to avoid the variation of other interfacial parameters. Conversely, when the objective is to characterize a chemical or physicochemical modification, it should be taken care to work with the same surface topography throughout. This objective seems fairly achievable at the micro-scale but more challenging at the nanoscale.

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Declaration of interest

None.

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