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ABSTRACTS BOOK

Biocorrosion of mild steel in drinking water conditions and disinfection

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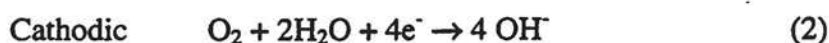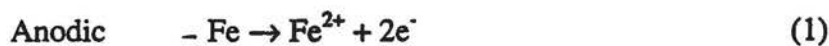4700 Braga, PORTUGAL

Introduction

Corrosion in drinking water distribution system is a costly phenomenon, mainly due to the replacement of altered pipes. Bio-corrosion is also a problem in term of public health because of the suspected protection brought by the corroded surface to potentially harmful microorganisms, especially bacteria. The protection effect of corrosion is particularly relevant in the presence of disinfectant. In drinking water are present the conditions leading to microbialy induced corrosion: bacteria and metal-containing substrata joined closely together as biofilm attached to distribution system pipe walls. Despite the economical interest of this subject and the need of understanding of the phenomenon, the relationships between biofilm and corrosion in drinking water distribution systems are largely unknown. Most of the published studies on bio-corrosion and disinfection are empirical from observations made on parts of pipe of distribution networks. This situation is probably due to the difficulty to distinguish and control the different parameters of this phenomenon: the surface area, the fixed and suspended corrosion products, the bacterial populations fixed and suspended, and the relationships existing between these parameters and between each of them and the added oxidant. Our research project therefore focuses on the corrosion of steel in conditions relevant to drinking water distribution system. Using a laboratory reactor allowing us to form and to sample biofilms on different surfaces with actual tap water bacterial populations under turbulent flow regime, we studied the biofilm-corrosion relationships and the disinfection of biofilm grown in corroded environment. This paper presents results obtained on these two aspects following a presentation of the corrosion phenomenon in drinking water distribution systems and the reactor we used to study it.
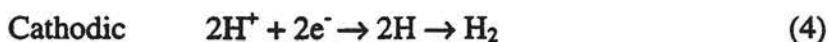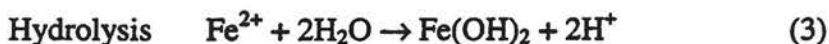
Biofilm and Biocorrosion

Microbialy influenced corrosion (MIC) is recognised as an important aggravating factor in the corrosion of metallic pipes. Biofilms are of central importance in this phenomenon by modifying electrochemical processes, notably through their heterogeneity both in the horizontal dimension (patchiness) and in the vertical one (existence of aerobic and anaerobic zones) and their metabolic activities. Horizontal and vertical heterogeneity is one of the features of biofilms on drinking water distribution pipe walls. In actual distribution systems as they could have been observed, biofilms

appeared indeed constituted of microcolonies sparsely distributed on pipe wall surfaces (Ridgway and Olson, 1981; Sly et al., 1988). The thickness of such a biofilm equals the height of the bacterial colonies, in the order of a few tens of micrometers. However, a gradient of oxygen can form between the bulk-colony interface and the base of the colony, i.e. the substratum surface (Patel and Bott, 1991; Xu et al, 1998). This organisation leads to the formation of differential aeration cells between the base of the bacterial colonies where anodes develop and the surrounding oxygenated non-colonised surface becomes cathodic (Little et al, 1990). The anodic and cathodic reactions are as follows:

$$\text{Anodic} \qquad - Fe \rightarrow Fe^{2+} + 2e^- \qquad\qquad (1)$$

$$\text{Cathodic} \qquad O_2 + 2H_2O + 4e^- \rightarrow 4\,OH^- \qquad (2)$$

Then, hydrolysis of the ferrous ions takes place (eq. 3) coupled with a cathodic reaction producing H_2 (eq. 4). The acidification of the anodic zone enhances the corrosion.

$$\text{Hydrolysis} \qquad Fe^{2+} + 2H_2O \rightarrow Fe(OH)_2 + 2H^+ \qquad (3)$$

$$\text{Cathodic} \qquad 2H^+ + 2e^- \rightarrow 2H \rightarrow H_2 \qquad (4)$$

In presence of free chlorine, ferrous iron can also be oxidised into ferric ions:

$$2Fe^{2+} + HOCl + H^+ \rightarrow 2Fe^{3+} + Cl^- + H_2O \qquad (5)$$

Ferrous and ferric hydroxides are bound to the extracellular polymeric substances (EPS) constitutive of the biofilm matrix and lead to the formation of others concentration cells that participate in the corrosion process. Since the water bulk concentration of oxygen is an essential parameter in the corrosion process and particularly its diffusion rate to the surface, it may be useful to note that in distribution systems the flow regime is not constant. Variations of flow occur along the day with peaks of consumption in the early morning and in the evening, and also depending on the location and diameter of the pipes in which the water is transported. The diffusion of oxygen towards the pipe wall is radically different in turbulent flow regime and in laminar flow regime or stagnant flow (de Beer et al, 1996), and then is the availability of oxygen for cathodic depolarization. On a bacteriological point of view, it has been demonstrated that water stagnation in segments of the distribution system (dead-ends) has profound and adverse impacts on the quality of potable water (LeChevallier et al, 1987).

The formation of anaerobic zones underneath the colonies is due to the oxygen consumption by the active aerobic bacteria forming the biofilm of this oxygenated medium. The presence of anaerobic zones in the biofilm procures the right conditions for the proliferation of anaerobic bacteria (Hamilton, 1995; Lee et al., 1995). Aerobic and facultative anaerobic bacteria can produce metabolic compounds suitable as nutrients for the strict anaerobic bacteria and create physiochemical conditions necessary to their growth (Hamilton, 1995). They also can produce organic acids that promote corrosion. The bacteria of choice in the field of biocorrosion are strict anaerobic bacteria

capable of reducing sulphate and producing sulphides which greatly stimulate corrosion, such as *Desulfovibrio* spp (Hamilton, 1995). The SRB have been shown to have an important effect on the corrosion phenomenon, particularly in process water (Weber and Knopf, 1996). However, if the development of microbially influenced corrosion by sulphate reducing bacteria is not in opposition with an aerobic environment such as drinking water, as explained above, we do not know any example of sulphate reducing bacteria isolated from this environment. Percival *et al* (1998) failed to identify SRBs on stainless steel coupons placed for 12 months in mains water. That does not mean however that there are not present. It is very difficult to sample actual distribution system biofilm in the conditions allowing for the isolation of SRB, already delicate to cultivate at the laboratory. Perhaps the low concentration of sulphate in potable water does not create a particularly suitable medium for the growth of SRBs either. Whatsoever, sulphate reducing bacteria and other anaerobic are not necessary for biocorrosion to happen and all types of bacteria able to grow biofilm can affect electrochemical processes.

It is not known what is the impact of corrosion of drinking water distribution system pipes on the bacterial populations present there and the precise influence of this specific type of biofilm on corrosion. Microbially influenced corrosion is not a one-way process; bacterial films affect corrosion, as seen above, and protection of metal from corrosion under certain conditions have been reported (Pedersen and Hermansson, 1991). Corrosion affects disinfection efficacy and may generate physical shelter to bacteria (LeChevallier *et al.*, 1987; LeChevallier *et al.*, 1993). A simple survey of the tap water quality of an old district of the city of Braga, Portugal, showed the presence of total iron in the bulk water phase at concentrations between 0 and 6 mg L^{-1}. The concentration of residual chlorine was negatively related to this of iron (figure 1). Gauthier *et al* (1997) showed that the iron content of the particulate matter of a distribution system was increasing during the transport in pipeworks probably as a consequence of corrosion inside the system.
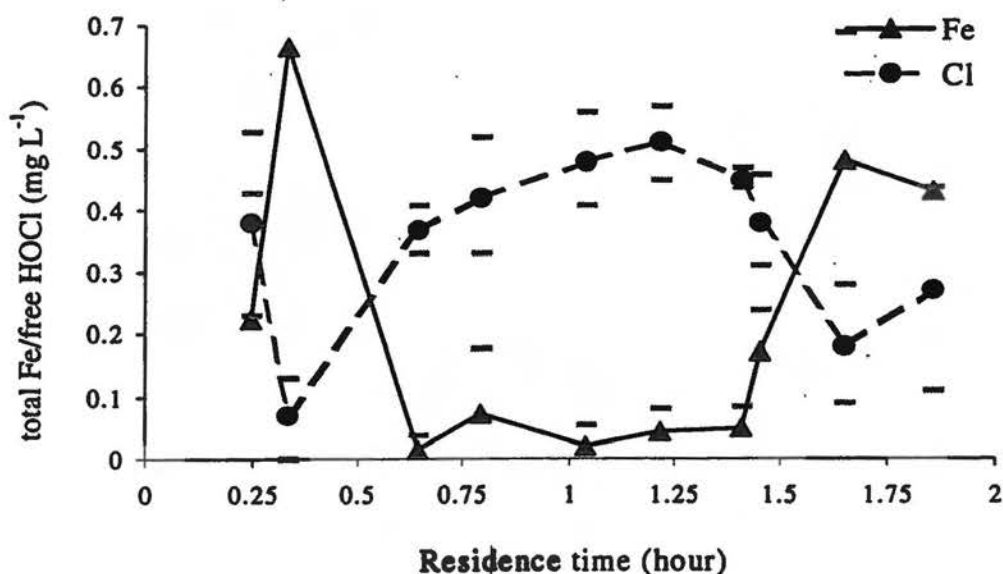
Figure 1: Total iron and free chlorine concentrations versus residence time in bulk water of an old district of the city of Braga, Portugal.

Laboratory system to study biocorrosion in DWDS conditions

The biofilm found on the drinking water distribution system pipe walls is formed with the bacteria released from the treatment plant and from the erosion and sloughing of biofilm developing in other locations on the pipes (Block *et al.*, 1993a; Laurent *et al.*, 1993; LeChevallier *et al.*, 1987; Lévi et Joret, 1990; Servais *et al.*, 1992b; Dunkelberger et Carey, 1991; Mathieu *et al.*, 1993). These bacteria were able to survive the disinfection applied at the treatment plant and eventually in the distribution system itself, and to grow in an oligotrophic medium. It has been shown that biofilms are developing even in so-called biological stable water, *i.e.* in the presence of very low load of biodegradable organic matter (Sibille *et al.*, 1997). The pressure brought by the harsh conditions of the medium selects for particularly resistant bacteria compared to laboratory kept bacteria. In order to work in conditions relevant to drinking water distribution systems, it was thus important to grow biofilms with tap water bacteria. We therefore designed a laboratory reactor plugged on tap and allowing us to study biofilm-corrosion relationships while controlling important parameters such as hydrodynamic, nutrient load, presence/absence of disinfectant, temperature, pH, etc.... The laboratory reactor presented in Figure 2 is described elsewhere (Morin and Camper, 1997; Morin *et al*, 1996) for its first part upstream the biofilm apparatus. It allows for the distribution of drinking water without chlorine residual and with a minimum concentration of utilisable organic carbon and other nutrients. The apparatus to grow biofilms is made of two flow cells in serie in a recirculation loop. This is a perfectly mixed continuously fed reactor with a residence time of two hours. Ten removable coupons disposed on the flow cells are used to develop biofilms on diverse material without emptying the reactor. The temperature is maintained at 23°C.
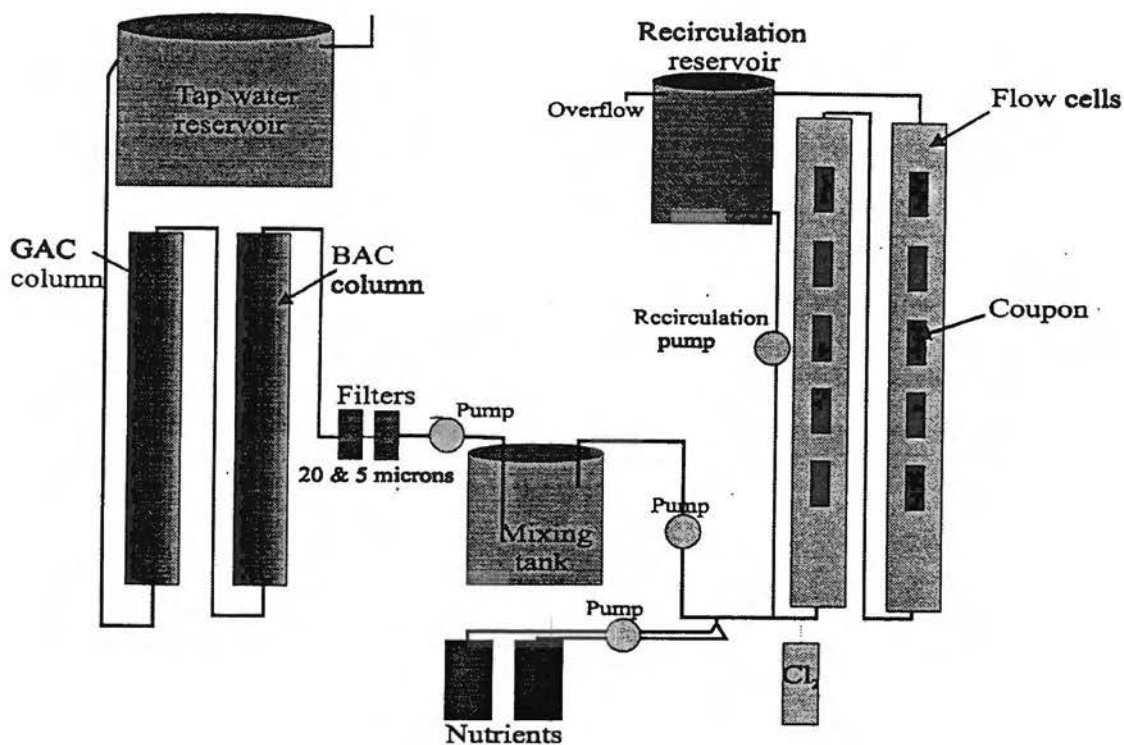
Figure 2: Laboratory system used to grow biofilm under conditions relevant to drinking water distribution systems.

We used the laboratory scale biofilm reactor to study the crossed influences existing between biofilm and corrosion, and between these two components and a strong and widely used oxidant in drinking water, chlorine. Two reactors were run in parallel with coupons of different nature in each: mild steel and metacrylate. Because biofilm reaches pseudo-steady state after six days of run (continuous feeding of nutrients and tap water, recirculation at 0.3 m s^{-1}, turbulent flow), each experiment started after seven days. Biofilm was treated by scraping the coupons and homogenisation, and analysed for heterotrophic plate count (HPC) bacteria numerated as unit forming colony (UFC), total bacteria through DAPI staining and epifluorescence microscopy and total iron (standard methods) and weight loss.

Biofilm-corrosion relationships

When mild steel coupons were placed in the biofilm reactor and this one run for a week minimum, important corrosion appeared at their surface under the form of nodules. It covered the major part of the surface area, leaving apparently untouched locations, and presented the specific orange colour of ferric hydroxide. However, dark corrosion layer(s), supposedly of magnetite and ferrous hydroxide, appeared when scraped. The biofilm scraped from the metacrylate and mild steel coupon surface was composed of a mix of HPC bacteria from the tap water. No statistical differences

were found between the densities of the biofilms grown on the two surfaces, despite the presence of larger surface area developed in the case of mild steel coupon due to the corrosion. The size of the bacteria measured from microscope photographs after DAPI staining was greater for the bacteria composing the biofilm grown on metacrylate than for those from the mild steel coupons. This difference in size for bacterial populations that are coming from the same source is an indication on the physiological state of the microorganisms and consequently on the environmental conditions they are living in. Under stress, bacterial cells undergo physical and physiological changes such as alteration of the membrane composition and size decrease, among many others. This participates to a survival strategy allowing the bacteria to grow and multiply in stressing environments such as... drinking water (Morin et al, 1997). In the conditions of our experiments, these results means that the bacteria growing in biofilm on a corroded surface were more stressed than if developing on metacrylate. Another indicator, the viability of the bacteria (UFC cm^{-2} * 100 / total bacteria cm^{-2}), for the biofilm populations grown on the two surface areas showed also that the bacteria associated with the metallic corrosion were not as healthy as those developing on metacrylate. That may be due to negative chemical interactions of the corrosion products with the attached bacteria or/and to the presence of these bacteria under the corrosion layers that would act like a diffusion barrier for the oxygen, the nutrients and the bacterial metabolites. Under the microscope the surface of the corrosion appeared to support bacteria, singly or in microcolonies (Figure 4) as already described for an actual drinking water distribution system (Ridgway and Olson, 1981; Sly et al, 1988). Percival et al (1998) showed that the bacteria growing on stainless steel coupons in mains water were associated with crevices and embedded in EPS. Disinfecting the coupon with sodium hypochlorite (30 mg free chlorine per litre for 10 minutes) showed very little detachment effect on the bacterial population attached on a corroded coupon. After one hour contact time almost all the bacteria were gone. Almost all the bacteria, but not all. This illustrates the difficulty to control biofilm on such a surface (Chen et al., 1993). After application of the disinfectant and scraping of the coupon we numerated 7×10^6 bacterial cells per cm^2 within the corroded layers. These results show that a large number of the bacteria are located in or under the corrosion layers that may be in that case a diffusion barrier as explained above. On the other hand, the corrosion provides bacteria with physical protection, particularly against chemical disinfection. This could lead to public health related problems if the results of LeChevallier et al. (1987), who found that coliform bacteria where preferentially situated in the ferrous tubercles of the studied drinking water distribution network, were to be generalised. Percival et al (1998) pointed out the potential public health threat represented by the opportunistic pathogens isolated form mains water biofilm. The protection brought to the bacteria by the corrosion layers obviously plays an important role in the biofilm resistance to disinfection (LeChevallier et al, 1987).

Biocorrosion and chlorine disinfection

The problem of disinfection experiments of bacterial population often resides in the disinfectant demand of the system that consumes the chemical before it can reach and act against its target, the bacteria. This problem is particularly true for chlorine due to its very high reactivity that makes it oxidise more or less everything it is in contact with. Consumption of the oxidant is the fact of the bulk water organic and inorganic particulate and dissolved matter, including bacteria, and of the pipe wall surface elements, such as the substratum material, the biofilm and associated particulate, the corrosion products (Chen et al, 1993). It was therefore important to keep that into account while studying the disinfection of our corroded system. We performed disinfection with sodium hypochlorite at residual concentrations up to 0.8 mg L^{-1} (free chlorine) in continuous for 48 hours. The residual chlorine concentrations used in these experiments are comparable to those encountered in actual distribution systems (Donlan et al, 1994). The chlorine demand of the corroded reactor was important and represented approximately 90% of the inlet chlorine dose, to compare with the 15-30% of the inlet

chlorine dose consumed in the non-corroded reactor. No effect of the added free chlorine was measured on the rate of corrosion as monitored by weight loss and total iron concentration in the bulk phase. Frateur et al (1999) showed also no involvement of free chlorine in the cathodic process coupled with iron dissolution on cast iron pipe, but only in the oxidant demand. After the first few hours of chlorination the initial chlorine demand of the system was satisfied and did not represent a negative parameter of the disinfection efficacy. It is worth noticing that the chlorine demand of the system implied the oxidation of some part of the biofilm and thus participated in the overall disinfection process.

The results of the analysis of biofilm for total bacteria and UFC counts presented in Figure 6 for two free chlorine concentrations illustrate the protection brought to the bacteria by the presence of corrosion compared to the non-corroded reactor.
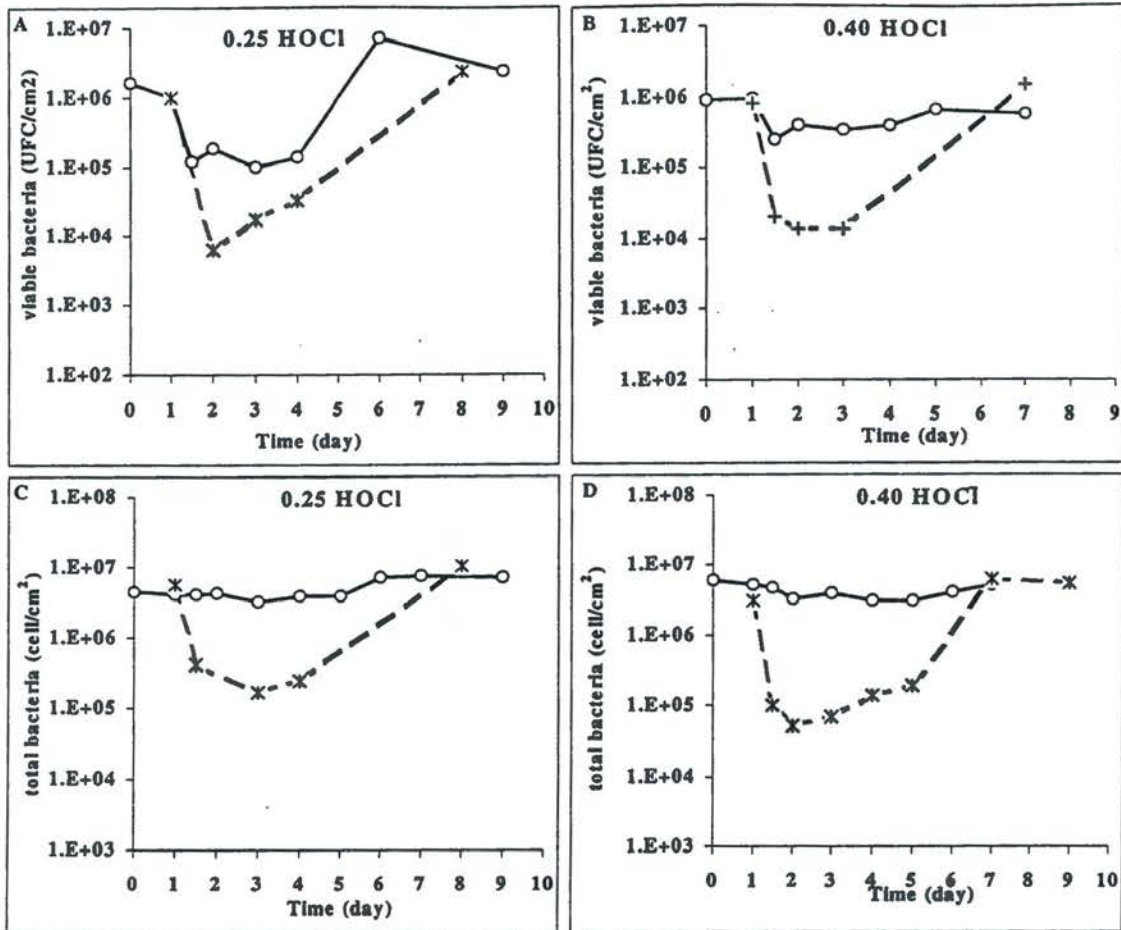
Figure 6: Results of the analysis of biofilm for total bacteria and CFU counts for 0.25 and 0.40 mgL⁻¹ free chlorine concentrations applied for two days. Plain lines represent the results for the biofilm grown on mild steel coupons and dashed lines for the biofilm grown on metacrylate coupons.

The first point to stress from the results presented on Figure 6 is that, whatever the surface characteristics, it is hardly possible to remove biofilm from the drinking water distribution system pipe walls by chemical means at the concentrations allowed by the legislation. Numerous works demonstrated that difficulty (LeChevallier *et al*, 1987; Mathieu *et al*, 1992 & 1993; Neden *et al*, 1992). The efficacy of the disinfection decreases when the bacteria have the possibility to be physically protected on the surface through the production of extra cellular polymer and the presence of roughness, cavities, etc... and when the chlorine demand of the environment is important. We can clearly notice the different impact of sodium hypochlorite treatment on the viability (Fig. 6A & 6B) and on the detachment (Fig. 6C & 6D) of the biofilms developed on the metal coupons or on the inert coupons. The effect of residual free chlorine is minimal in the case of corroded surface and pronounced for bacterial populations grown on metacrylate. Decay and detachment rates as calculated from UFC and total bacteria results, respectively, are presented in Table 1.

	Mild Steel [chlor.]r = 0.25	Mild Steel [chlor.]r = 0.40	Control [chlor.]r = 0.25	Control [chlor.]r = 0.40
Decay (%)	89.8	73.8	99.3	99.5
Decay rate (day^{-1})	1.83	0.58	2.20	1.14
Detachment (%)	23.3	41.8	96.8	98.3
Detachment rate (day^{-1})	0.057	0.21	0.5	2.21

Table 1: Decay and detachment rates calculated from UFC and total bacteria results for the reactor biofilms grown on mild steel and on metacrylate (control) coupons. [chlor.]r = residual chlorine concentration in the reactor effluent in mg L^{-1}.

The bactericide effect of the disinfectant is more important (99% versus less than 90%) and slightly faster on metacrylate attached bacteria than on corroded coupon associated bacteria. However, the biofilm on the steel coupons did not seem to be as protected when we look at its viability rather than at its detachment, i.e. the effect of the disinfectant on the viability of the bacteria is more pronounced. That does not mean, however, that the bacteria are dead. They can be only slightly injured, enough to forbid them to multiply on agar medium but not to grow in their original medium (Camper and McFeters, 1979; Walsh and Bissonnette, 1989). The detachment rate due to the application of chlorine in the reactors was ten times larger for the biofilm on the metacrylate surface than for the attached bacterial population on the metal coupon. As it appears, any detachment of bacteria from the metal coupons is not even evident. The apparent decrease of bacterial numbers on the surface may be an artefact due to the chlorination. Saby *et al* (1997) showed that the presence of free chlorine was sensibly decreasing the staining of bacteria with DAPI compared to a non-chlorinated suspension. The blue colour of the stained bacteria was fading and the cells increasingly more difficult to see (Saby *et al*, 1997). In our experiments, the bacteria associated with the corrosion layers on the metal ocupons were already particularly small even before disinfection. It is therefore likely that a certain percentage of the total bacteria were not numerated. Chen *et al* (1993) experienced a larger detachment of biofilm from mild steel slides in an annular reactor treated with 4 mg L^{-1} monochloramine for one hour. This may be due to the growth conditions of the bacteria and the structure of the developed mono-specie biofilm. Another reason could be the supposed better detachment effect of monochloramine on biofilm compared to chlorine (Griebe *et al*, 1993; LeChevallier *et al*, 1998). In the contrary, Morin and Camper (1997) found that chlorine was more efficient in killing and detaching biofilm bacteria in simulated drinking water distribution systems. From these results and the experiment related above (Biofilm - corrosion relationships) that showed

the important resistance of biofilm bacteria attached to the external corroded layer surface, we can hypothesise that only these bacteria are reached by the disinfectant. The difference between the two types of biofilms in their response to the chlorination is more important in term of detachment (total bacteria results) than in term of death (UFC results). For that reason, one can imagine that when the chlorine CxT (concentration by time) is high enough it can affect the viability of the bacteria sheltered in the corroded layers without detaching the biofilm grown there. It is known that the disinfection (decrease of viable bacteria numbers) of biofilm bacteria is possible without detaching them from the surface (Srinivasan et al, 1995).

Conclusions

Drinking water distribution networks are complex systems in which numerous parameters are involved, most of them hardly controllable. Among those parameters, disinfectant, biofilm and corrosion are directly linked to the quality and the quantity of the water being distributed to the tap. The interactions between them are little known. This paper presented some results related to this problem. Conclusions are as follows:

- Biofilm grown under conditions relevant to drinking water distribution systems in presence of solid corrosion products at the surface of mild steel coupons appear to develop mainly under or within the layers of corrosion.

- Evidences of a detrimental effect of corrosion products on the bacterial populations have been found. Bacteria grown in these conditions are smaller and not growing as well on solid agar medium as those developed on metacrylate coupons.

- The presence of corrosion products leads to a high chlorine demand that participates in the noted little efficiency of sodium hypochlorite against the biofilm.

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