EFFECT OF ARSENIC ON MICROORGANISMS AND BIOFILMS IN MIC OF WATER NETWORK MATERIALS

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INTRODUCTION

Microorganisms in natural and artificial aquatic environments attach to wet surfaces to form a biofilm (Dexter, 2003), which causes problems such as microbially induced corrosion (MIC), increased resistance to biocides and persistence of pathogenic species. Arsenic (As) is being increasingly detected in distribution water services, generating serious sanitary and social problems throughout the Planet (Pontius et al. 1994; Masud Karim 2000). The existence of bacterial species able to grow in the presence of high concentrations of this chemical is well known (Oremland and Stolz 2005).

The development of molecular techniques has allowed the study of microbial communities (Muyzer 1999). Among these techniques denaturing gradient gel electrophoresis (DGGE) is commonly used for genetic fingerprint analysis of microbial community composition, diversity and dynamics (Green et al. 2009). Electron microscopy techniques such as scanning electron microscopy (SEM) and environmental SEM (ESEM) have been important for high resolution visualization of bacterial biofilm outer surfaces.

The main purpose of this work was to shed light on the effect of As(V) on the sessile bacterial communities produced on four substrata used in water distribution systems and their correlation with susceptibility to MIC of these substrata, using molecular biology and microscope techniques.

MATERIALS AND METHODS

Experimental set-up. Two laboratory water distribution systems containing test coupons with La Plata drinking water flowing at laminar flux were used (Rosales et al., 2011) and 5mg $.L^{-1}$ As(V) was added to one of them. The coupons (10 mm x 10 mm x 0.2 mm) were made of materials commonly used for water distribution, commercial iron (Fe), Zn, copper (HIDRO-BRONZ®) and polypropylene (PP). The surface was finished with emery paper up to 1,000 grade. After 45 days, a cell containing 7 coupons of each material was extracted from each loop. This procedure was repeated in 7 independent runs (series).

Microscopy of biofilm and corrosion attack. A stereoscopic microscope and scanning electron microscopy (SEM) were used. Energy Dispersive Spectroscopy (EDS) was applied for elemental analyses. Samples for SEM were fixed, dehydrated, critical point dried and surface coated. Samples were also treated with a commercial pickling solution prior to observation. The relationship between surface attack and biofilm elemental composition was also analyzed.

Microbial characterization. 1L water from each tank was filtered through 0.22 µm sterile membrane at the end of a test series. Biofilm, EPS and corrosion products were removed from 4 coupons by scraping into 1mL sterile physiological solution. One suspension was used to analyze tolerance of the sessile bacterial community to As(V) by culturing in nutrient broth with 50 - 1,000mg.L^{-1} As(V); the remaining 3 were combined for DNA extraction. Total and culturable sessile and total planktonic DNA was extracted using a commercial kit. DNA was amplified by the polymerase chain reaction (PCR) using 341F with a GC clamp and 907R primers (Muyzer 1999). DGGE was performed in a 6 % w/v polyacrylamide gel with a 30-70 % denaturing gradient (100 % denaturant is 7 M urea and 40 % v/v formamide). Electrophoresis was performed in TAE buffer for 16 h at 60 ºC, at 100 V. Gels were stained with SybrGold for 40 min and observed in a UV transilluminator. Band position and intensities of the gels were normalized using appropriate software.

RESULTS AND DISCUSSION

Biofilm and corrosion attack. From the beginning, heterogeneously distributed microbial colonization was seen on all exposed materials. With time, increasing amounts of corrosion products and solids from the drinking water appeared, mixed with the biofilms and EPS. This was seen especially on Fe and Zn samples. At the end of each test series the

deposits showed a very heterogeneous distribution on all test samples. Figure 1 shows SEM micrographs for each material in both circuits.

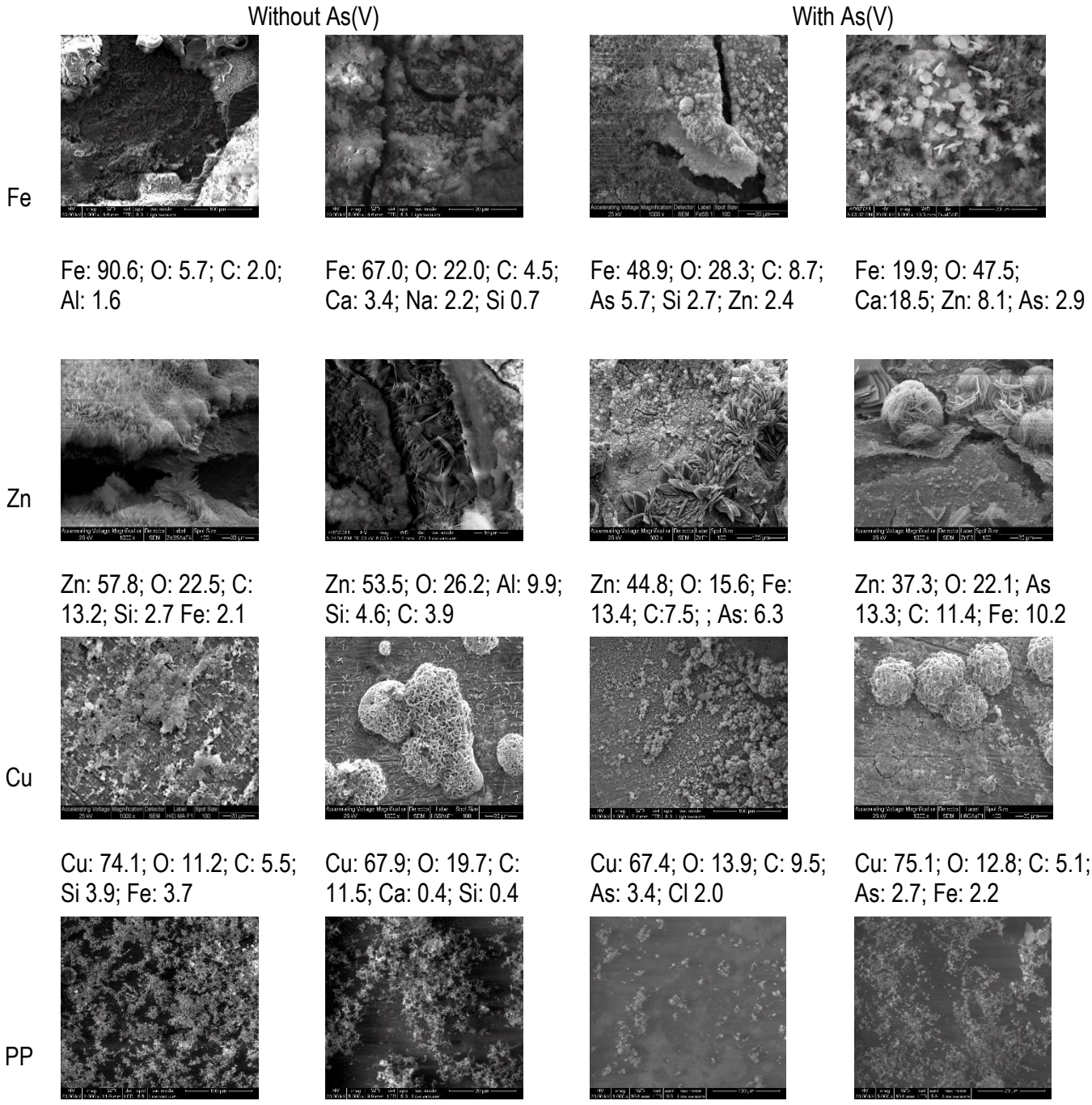

Figure 1: SEM and EDS surface analysis of coupons exposed in both circuits.

Coupons in the presence of As(V) showed higher deposit accumulation on Fe and Zn samples. Arsenic was only detected in EDS in those areas covered by biofilms. The surface failures detected on PP near microbial cells were not attributed to microbial biodeterioration, but to the process of thermocompression applied. The attack morphology observed by SEM/ESEM after removal of the deposits was characteristic of localized MIC. This morphology seemed to depend more on the substratum and metallurgical structure than on biofilm characteristics. Fe and Zn showed hemispherical localized attack with corrosion products accumulating at the bottom, while localized attack on Cu revealed the alloy crystallography. The same results were observed in the presence of As.

Microbial characterization. Direct PCR-DGGE of the total planktonic and sessile DNA allowed a description of the structure of microbial communities present in the water and in the biofilms. The number of DGGE bands was used as an indication of the number of dominant bacterial groups (Portillo and Gonzalez, 2008). Figure 2 illustrates the DGGE band patterns of planktonic communities present in both circuits in 7 independent runs. Each sample produced a distinctive molecular profile with a different number of bands of varying intensities, different from the profiles generated by any other sample. The different profiles at the end of each experiment indicated that the bacterial community was affected qualitatively (band position) and quantitatively (band intensity) by the presence of arsenic in the water. The diversity of the bacterial community was very variable. Several runs showed relatively high numbers, while other samples exhibited very few bands.

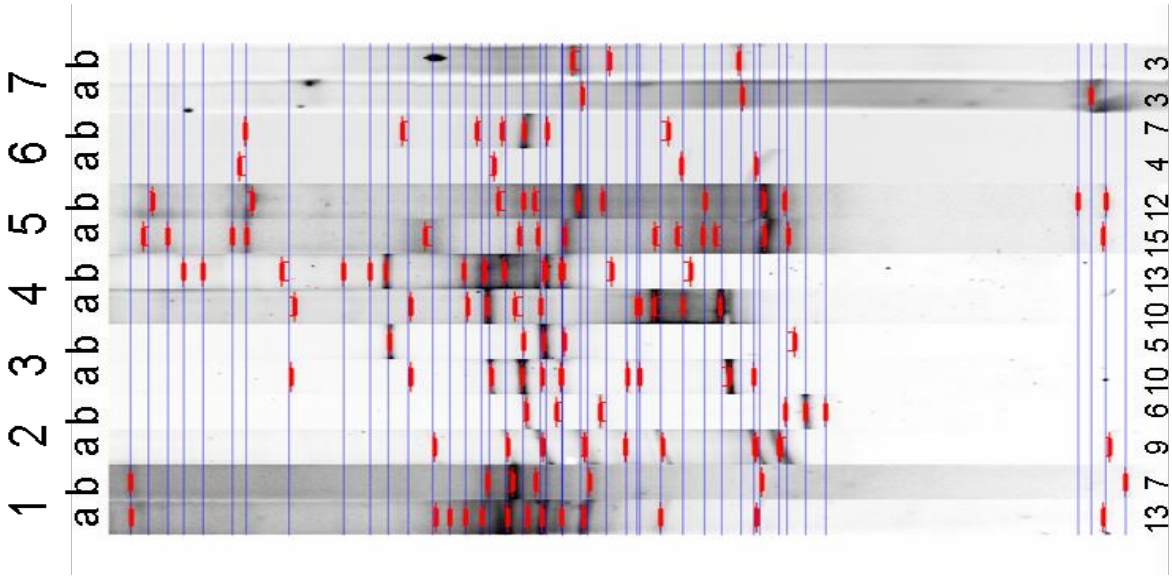

Figure 2 - DGGE fingerprint gel of 16S rRNA from DNA of the planktonic communities in seven independent runs in both circuits. On left, **1** to **7**: number of run; **a**: without As, **b**: with As; On right: number of bands in each lane.

Profiles of the sessile communities formed on each material were obtained. Only series 1 and 7 yielded enough DNA from biofilms formed on all the substrata. In the other experiments some biofilms, particularly those formed on PP and Cu, contained too low a number of microorganisms for DNA extraction and PCR amplification. The profiles of the bacterial sessile communities growing on each substratum are shown in Figure 3. The community profiles on each substratum in the absence (lanes “a”) or in the presence (lanes “b”) of As(V) showed no significant difference (with the exception of biofilms on Zn in experiment 1). Thus, it could be concluded that the nature of the substratum is a more important factor for the establishment of the sessile community than the presence of arsenic.

When biofilms from metal coupons were cultured at different As levels, even at high As concentrations, the number and intensity of the bands in the cultures were higher than in the original biofilm. This is due to the increase in biomass produced by culture. The diversity of the culturable heterotrophic fraction of the bacterial community has been analyzed in several papers. While some authors found that the presence of contaminant decreases the culturable bacterial diversity, other authors agree that it increases it (Rasmussen and Sörensen , 2001; Dell´Amico et al., 2008). Biofilm formation allows microorganisms to survive in the presence of contaminants (Shirtliff et al., 2002). In our case, this hypothesis is confirmed by the higher bacterial counts and more compact and thicker biofilms formed in the presence of As(V).

CONCLUSIONS

After exposure, the metal samples revealed higher colonization and corrosion product accumulation in the presence than in the absence of As(V). There were differences between the 7 metal replicates in all the test series. EDX analyses revealed the presence of As on the accumulated deposits. The structure of the biofilms formed on Fe and Zn was independent of the presence of As. The metal attack, seen after biofilms and corrosion products were removed, revealed

differences in alloy structure. Attack depended more on substratum susceptibility than biofilm composition and presence or absence of contaminants. PP did not show any biodeterioration.
The genetic profiles of planktonic communities obtained in the presence of As were different from those in its absence. The genetic diversity of biofilms was more affected by the nature and susceptibility to colonization of substrata than by the presence of As. As-tolerant bacteria able to grow at very high As(V) concentrations were found in biofilms in both circuits.

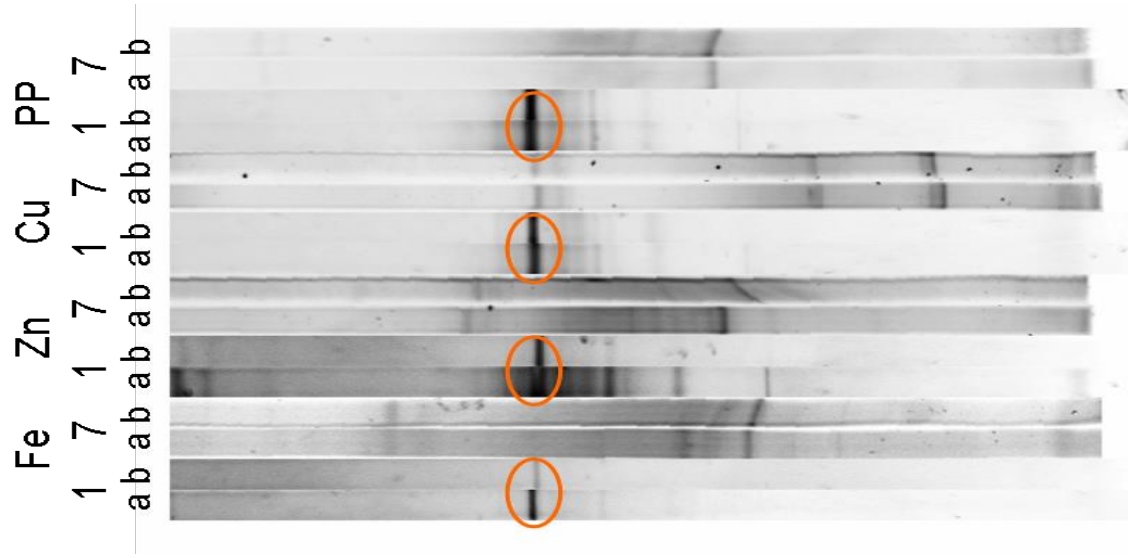

Figure 3 - DGGE fingerprint gel of 16S rRNA from DNA of sessile communities on Fe, Zn, Cu and PP; 1 and 7: selected runs for this analysis, a: circuits without As, b: circuits with As.

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