TRACKING MICROBIOLOGICALLY INDUCED CORROSION IN A SPENT FUEL ELEMENTS STORAGE POOL

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ABSTRACT

The storage of spent fuel elements from nuclear research reactors in demineralized water basins for long periods of times provides an environment where biofouling and biocorrosion could happen. Early detection is critical in order to counteract biocorrosion and prevent damages. Considering these factors, a program to monitor biofouling and biocorrosion by exposing of aluminum alloy AA6061 coupons at the water basin, as well as changes in the microbiology of the water, was established at one of the nuclear irradiated fuel storage facilities in Argentina. After one year, complex biofilms consisting in aerobic (including iron related bacteria) as well as anaerobic heterotrophic bacteria are developed on the coupons. Under black oxide-biofilm covered areas, incipient pitting corrosion was detected. No corrosion was detected after biofilm removal of clear zones. This work is the first experience of a systematic microbiological monitoring program of nuclear spent fuel facilities in Argentina.

1. Introduction

In Argentina, the spent fuel elements from nuclear research reactors, made mainly of aluminum alloy AA6061, are stored in de-mineralized water basins. This environment does not prevent the development of complex associations of microorganisms. These communities have been subject of increasing studies, both for their possible use in bioremediation programmes [1] as well as for their potential to induce biocorrosion [2,3,4].
Taking into account that AA6061 is susceptible to localized corrosion [5] including under-deposit corrosion induced by microorganisms [4], a microbiological and biocorrosion monitoring program was established at the Research Reactor Spent Fuel Elements Storage Facility (FACIRI). This program comprises the following tasks:

- Exposure of AA6061 coupons in the water basin at -8 and -15m depth for periods of 14-16 months, with the aim to evaluate biofouling and biocorrosion using different methodological approaches (specific culture media, scanning electron microscopy with energy dispersive X ray spectroscopy (SEM EDX).
- Design and construction of a sampler to take water samples in a sterile way at a radiological controlled area.
- Microbiological and chemical analysis of the water.
- Evaluation of methods to improve the microbiological quality of the water without interfering with the chemical characteristics. In particular, the use of ultraviolet radiation of 254 nm (UVC) is being tested at the laboratory both on pure cultures as well as on untreated water samples.

2. Experimental

2.1 Analysis of biofouling and biocorrosion on AA6061 coupons

On February 2014, AA6061 coupons with 8 equivalent sections each (Fig. 1) were immersed at -8 and -15 m on the basin at the FACIRI. They were exposed for 14 months and replaced for similar probes, which were also replaced for new ones after 16 months of exposure. Previously, all coupons were polished mechanically with emery paper p600, followed by immersion for 60 seconds in 10% NaOH solution at a temperature of between 60 and 70° C, rinse with distilled water at the same temperature, immersion for 10 seconds in 50% HNO_3 solution at room temperature, rinse with distilled water (temperature between 60 and 70 °C) and finally, dried with ethanol and hot air.
After the exposure, all the coupons were subject to the following treatments:

- One section of each coupon was scraped, the material obtained was suspended in Phosphate Saline Buffer (PBS) and serial dilutions were made using specific media (MAG laboratories) for heterotrophic aerobic bacteria (BAT), anaerobic bacteria (BAnT), iron depositing bacteria (BRH), acid producing bacteria (BPA) and sulphate reducing bacteria (BSR) in accordance with NACE standard TM194-1994.
- Two sections of each coupon were scraped to extract DNA and perform molecular analysis using MOBIO Power Water DNA isolation system.
- Two sections of each coupon were fixed with glutaldehide 2.5%, dehydrated with ethanol (Hamilton et al. 2003), gold coated and examined with a Philips 515 EDX PV9100 SEM to observe the biofilms. Afterwards, both biofilms and corrosion products were removed by immersion in phosphoric acid 35% and the surface was evaluated again using SEM EDX and optical microscopy in order to evaluate corrosion damage compatible with biocorrosion.

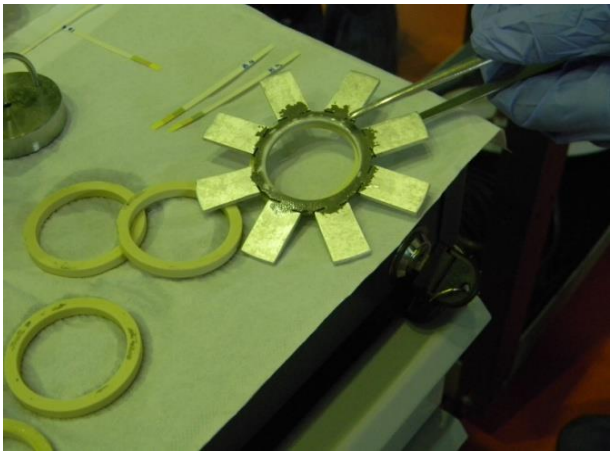


Fig 1. Coupons of AA6061 exposed to the water of a nuclear irradiated fuel storage facility.

2.2 Design and construction of a sampler

A sampler was constructed, consisting in a fixed body with a mobile part (that never leaves the nuclear facility) inside which a disposable sterile syringe of 60 ml is attached to the mobile part and use to take the sample as shown in Fig. 2.

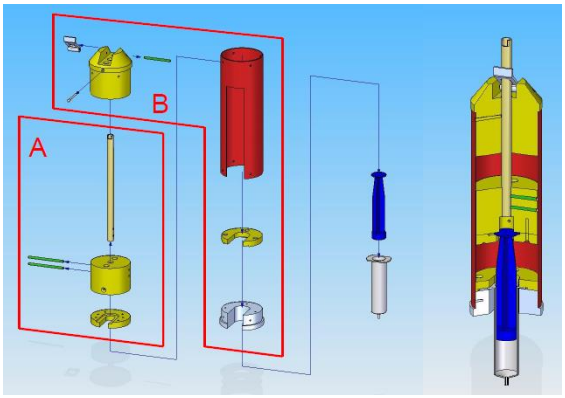


Fig 2. Sampler for taking sterile microbiological samples. (a) mobile (b) fixed

2.3 Analysis of water

Microbiological analysis of aerobic bacteria was performed by viable cell count in nutrient agar. Also, pH, conductivity and temperature of the water were measured.

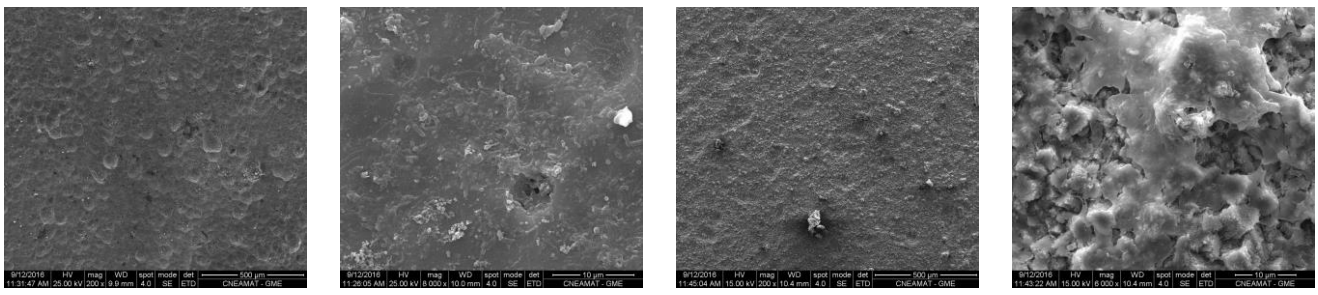
2.4 Study of the effect of UVC radiation on the bacterial population

The effect of UVC radiation (254nm) on water samples taken from -8m and on pure cultures of bacteria (isolated from the basin, maintained in nutrient agar plates grown in Nutrient Broth and resuspended in sterile distilled water to an initial optical density at 650 nm of 0.5) was evaluated. The irradiation protocol consisted in exposing the water/bacterial suspensions to a germicidal lamp for a maximum of 10 minutes, taking samples within that time period and analyzing the population using the plate count technique, consisting of preparing serial dilutions of the samples and plating aliquots of those dilutions on Nutritive Agar plates.

3. Results

3.1 Evaluation of biofouling and biocorrosion on AA6061 coupons

In all the analysed samples, images compatible with biofilms, bacteria and products of corrosion were observed (Fig 3). The presence of organic material was confirmed by EDX (data not shown). The samples showed zones covered by a black oxide with a higher biofilm content (Fig 3 c and d) and clear ones with a surface aspect similar to the appearence before exposure but covered with a thin biofilm (Fig 3 a and b). There were no differences between -8m and -15m.



(a) (b) (c) (d)

Fig 3. Biofilms on AA 6061 coupons. (a-b)Clear zones. (c-d) Black oxide covered zones.

Images compatible with incipient pitting corrosion (that could be induced by microorganisms) were observed under the black oxide-biofilm patches (Fig 4). No corrosion was detected after removing the biofilms from the clear zones.

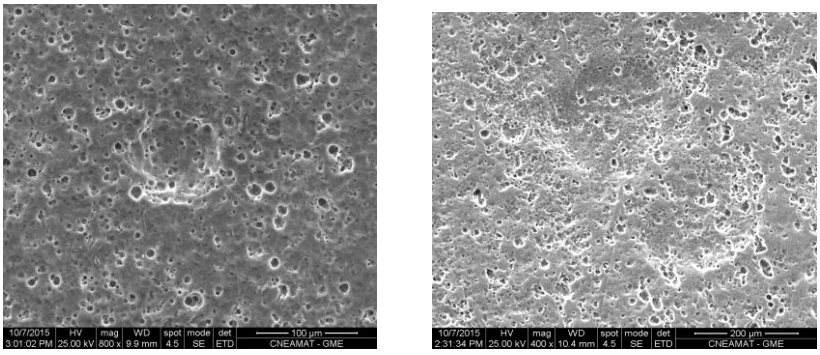


Fig 4. Example of corrosion under biofilm-oxide patches.

The analysis of the bacterial population by specific culture media showed presence of heterotrophic aerobic bacteria (BAT), anaerobic bacteria (BAnT) and iron depositing bacteria (BRH), both at -8m and at -15m. No acid producing bacteria (BPA) or sulphate reducing bacteria (BSR) were detected.
Due to the low amount of biofilm, and the limitations of the extraction kit, it was not possible to obtain DNA from the sessile community for further analysis. To improve DNA recovery from biofilms in order to perform molecular biology studies is one of the challenges for next years.

3.2 Analysis of the water

The conductivity of the water was usually less than 2 µS/cm (Fig 5) which is in accordance with the operations manual of the facility. During this period of time, pH was between 5 and 5.5 (Fig 7.). Temperature showed fluctuations related to seasonal changes (Fig 8.) and there was a difference of 1ºC between surface and -15m level.
Microbiological analysis indicated that the number of culturable aerobic bacteria was slightly higher in the samples taken at -15m than in those at -8m, remaining in all cases in the order of 10^3-10^4 Colony forming units (CFU)/ml.

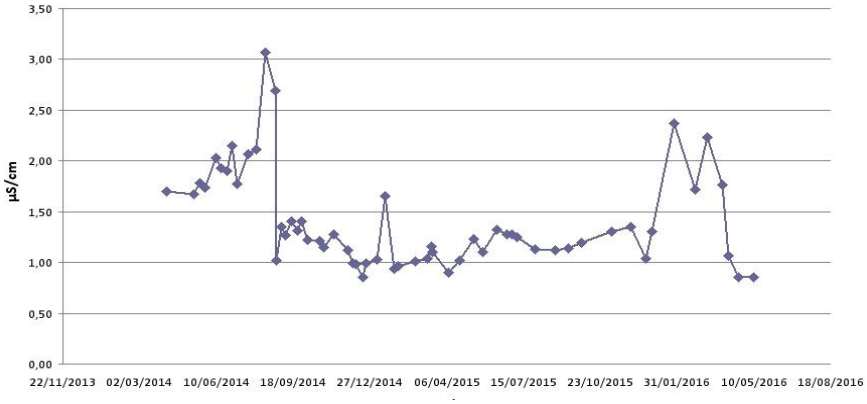


Fig 5. Conductivity of the FACIRI water basin.

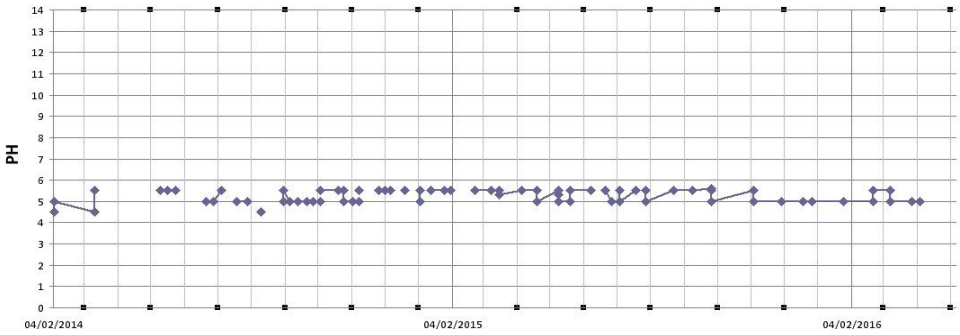


Fig 6. pH of the FACIRI water basin.

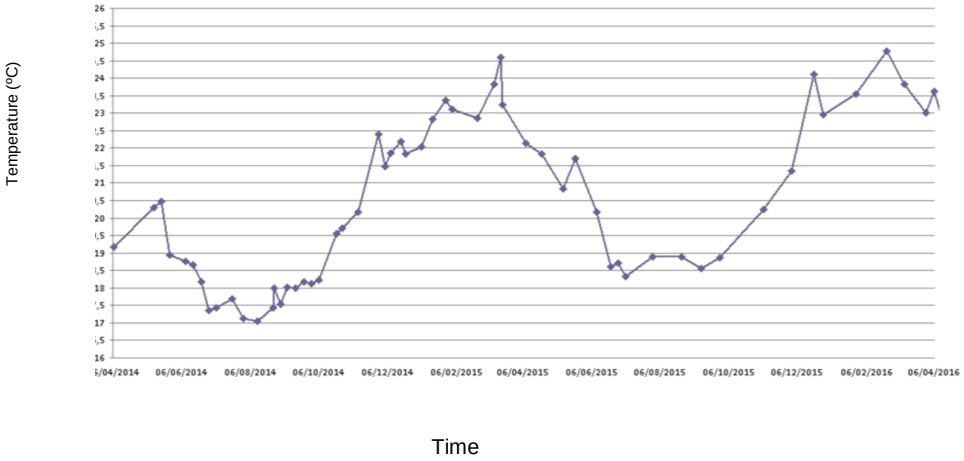


Fig 7. Temperature of the FACIRI water basin.

3.3 Study of the effect of UVC radiation on the bacterial population

After irradiation with UVC for 30 seconds of the water sample from -8m depth containing 2.6 10^3 CFU/ml, no living microorganisms were detected. These results suggest that UVC treatments could be a promissory approach to improve the water quality of the facility.

The exposure to UVC of a Gram negative strain isolated from the facility showed an abrupt drop in viability after the first minute. However, at longer irradiation times, when the measured survival fractions are low, the slope of the curve decreases (Fig 8.). This behavior could be attributed to the presence of a persistent subpopulation, as shown for bacteria exposed to sunlight [6].

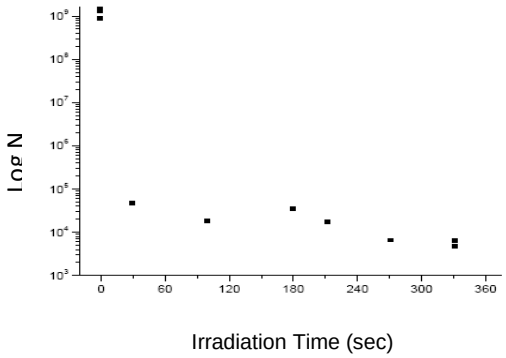


Fig 8. Gram negative strain exposed to UVC radiation

The irradiation of a Gram positive, sporulating strain isolated from the basin, exhibited a much slower decay followed also by a decrease on the slope after 6 minutes of irradiation

(Fig. 9). This difference could be possible related to the fact that the suspension contains a mixture of spores and vegetative cells. Also, the presence of a persistent subpopulation could not be discarded.

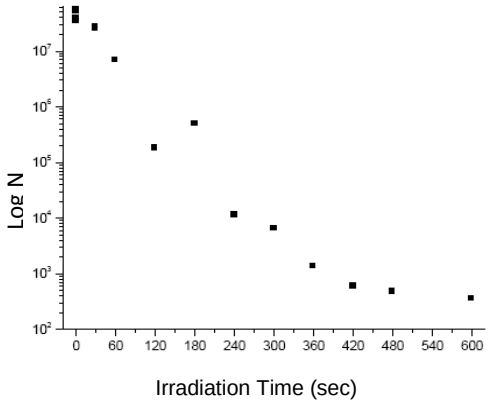


Figure 9. Gram positive strain exposed to UVC radiation

4. Discussion and Conclusions

After 14-16 months of exposure, both corrosion products and biofilms were observed in the AA6061 coupons submerged at -8 and -15m. Two different zones were detected in the coupons: clear areas, with an aspect similar to the one before exposure, covered with thin biofilm and dark areas showing globular oxides associated with more compact biofilm patches. Under the patches images compatible with pitting corrosion induced by microorganisms were observed. No corrosion was detected after removing the biofilms and oxides from the clear zones.

The analysis of the biofilms shows presence of microorganisms with the ability to precipitate iron, as well as microorganisms that produce exopolysaccharides. Both types of microorganisms could be associated with microbiological corrosion phenomena, particularly in materials susceptible to under deposit and galvanic corrosion such as aluminum alloys.

Taken into account that only a small proportion of the microorganisms can be detected by culture based techniques, the use of molecular biology could be an important tool. Unfortunately, the quantity of DNA obtained from the biofilm samples was below the detection limit of the techiques. We ascribe this to the low amount of biofilm, and limitations of the extraction kit.

The water parameters of the nuclear irradiated fuel storage facility show few changes during the year. The results obtained (both chemical and microbiological) are within the expected for the installation.

Finally, the use of ultraviolet radiation of 254 nm (UVC) against microorganisms from the facility showed promissory results and could be a way to improve water quality without the adding of chemical biocides to the system.

Acknowledgments

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5. References

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