Report on the outcomes of a Short-Term Scientific Mission

Action number: **COST Action CA20130**

Grantee name: **Clara Ortega Nieto**

Details of the STSM

Title: Evaluation of Metal Bionanohybrids and its Antimicrobial Effect over Different Surfaces as Additives or Coating Materials.

Start and end date: 22/07/2022 to 22/09/2022

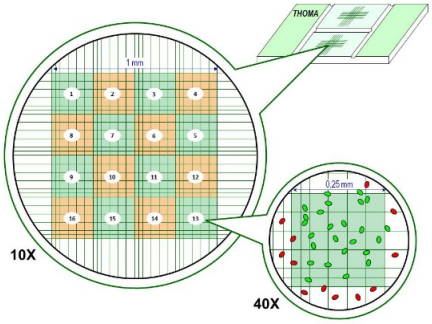
Description of the work carried out during the STSM

Description of the activities carried out during the STSM. Any deviations from the initial working plan shall also be described in this section (max. 500 words).

At the beginning of the STSM, I received a safety talk and a microbiology and sterile lab work training. It included how to work correctly in a sterile environment, how work with the equipment and how to prepare the materials.

After it, we have carried out the experimental work through 3 different steps:

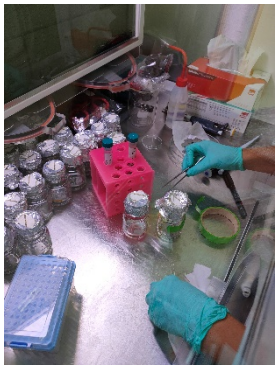
First, growth curves of the bacteria potentially involved in Microbiologically Influenced Corrosion (MIC) were made to study their evolution. For this purpose, we used strains of Sulphate Reducer Bacteria (SRB), Slime Forming Bacteria (SFB), which produce extracellular polymeric substances (EPS) and Acid Producer Bacteria (APB), provided by Endures B.V. and which have been isolated from different water natural environments. Each strain has been grown in an appropriate culture medium in a final volume of 5 mL. We made the growth curves from different initial bacteria concentration, to find the optimal one. We took samples at different times, from 0 to 50 hours, with 3 replicas per point, and counted them in a Thoma Cell Counting Chamber with a LEICA DM500 Microscope and a 40X objective.



Second, the antimicrobial properties of the bionanohybrids designed and synthetized in Jose Palomo's laboratory were tested against these strains. Growth curves were made in order to find if the materials inhibit their growth, comparing in each case with controls. The experiments were performed under the same conditions as the growth curves previously described but using an initial bacteria concentration of $1 \cdot 10^7$ cells/mL, adding 50 or 100 ppm of the additive to the mixture, and keeping the flasks at room temperature in an orbital shaker. We took different samples points between 0 and 70 hours in order to obtain the growth curves. Each sampling point was taken in triplicate.

Last, we tried to found if the materials, in presence of SRB bacteria, inhibit their growth and the formation of biofilm and MIC on the surface of steel coupons.

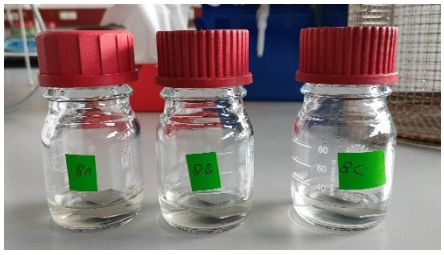
For this purpose, the best bionanohybrids against SRB were selected and small pieces of steel with a size of 2 cm x 2 cm were used. Before the experiments, the metal coupons were cleaned with distilled and sterile water and dried. Then, they were placed in the bottom of glass bottles with 20 mL of SRB medium, an adequate bacteria volume to obtain a concentration of order of magnitude 7 and 50 ppm of



the additive. The set-up was performed in the anaerobic chamber, to avoid the presence of oxygen. Each assay was performed in triplicates, and different controls were included in the experiment. Also, some bottles with media, additives and coupons but no bacteria were included as blanks.

To analyse the bacteria attachment to the surface of the coupons and their viability, the coupons were stained with a LIVE/DEAD BacLight Bacterial Viability Kit. After that, we used an Olympus BX51 Fluorescence Microscope, with a filter suitable for the fluorescence imaging.

Finally, the results were analysed and discussed. Tests with other metallic surfaces could not be performed due to lack of time.



Description of the STSM main achievements and planned follow-up activities

Description and assessment of whether the STSM achieved its planned goals and expected outcomes, including specific contribution to Action objective and deliverables, or publications resulting from the STSM. Agreed plans for future follow-up collaborations shall also be described in this section (max. 500 words).

Thanks to this STSM, it has been found that the bionanohybrids developed in Jose Palomo laboratory have antimicrobial efficiency against important MIC-causing bacteria.

In particular, the bionanohybrids are effective against Slime Former Bacteria and Sulphate Reducer Bacteria. It has been found that at concentrations of 50 ppm some bionanohybrids inhibit the growth of SFB in solution by as much as 98%. In addition, the bionanohybrids made of silver and copper are more efficient against SFB than those made only of copper. In experiments against SRB, it was found that the bionanohybrids which inhibited better the SFB growth, were slightly less efficient. This indicates that the differences in the composition of the bionanohybrids play a key role in their efficiency. Also, data suggested that some bionanohybrids work better against SRB in concentrations below 100 ppm. In this case, the best bionanohybrid showed an inhibition percentage of up to 98%. However, against Acid Producer Bacteria the bionanohybrids were not efficient. Evidence suggested that the low pH levels generated by bacteria destroyed the structural integrity of the bionanohybrids.

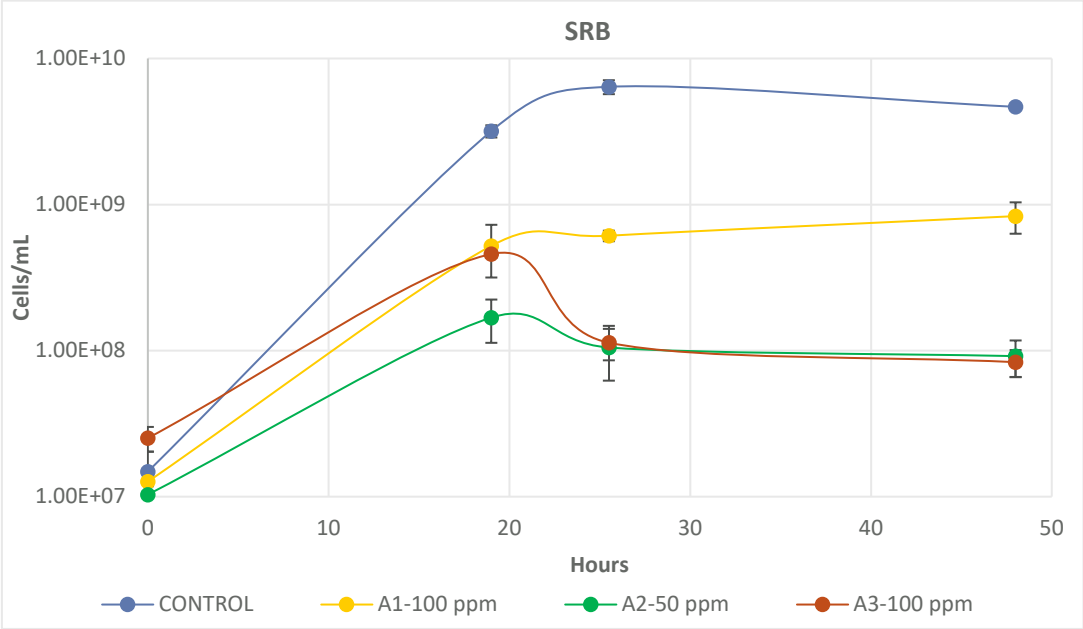


Figure 1: Example of some growth curves obtained for SRB with some bionanohybrids in different concentrations.

Regarding the steel coupons assay, the fluorescence imaging revealed that the presence of the bionanohybrids in the mixture reduced the SRB attachment to the coupons surface by up to 50%, with the best of them. However, the cell count in the liquid medium of coupons assay showed that bacterial populations grew to control levels in 48 hours, after an initial inhibition. This seems to indicate that the presence of metal coupons affects the antibacterial efficacy of bionanohybrids and stimulates the growth of SRB in solution.

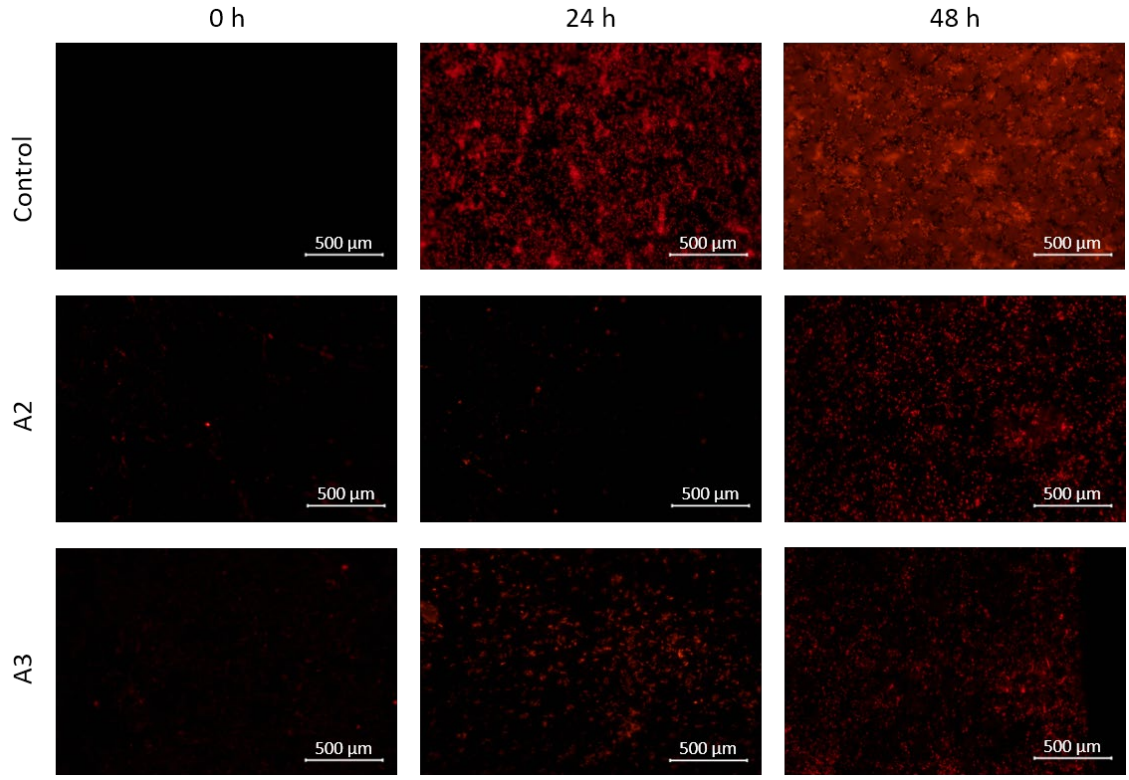


Figure 2: Examples of fluorescence images of coupons surface obtained for the experiment with different bionanohybrids and a control at 0, 24 and 48 hours.

The results obtained in this STSM have reached the planned objectives and have opened new doors for the development and design of the bionanohybrids. Future plans include redesigning the molecules to have a greater impact on MIC, finding an optimal attachment system for the molecules so that they serve to prevent and mitigate MIC on different susceptible surfaces, or further testing antimicrobial effects against other strains of different bacteria. In addition, good experimental results have been obtained and are being considered for a future paper.

Thus, the bionanohybrids may have a promising future in MIC prevention and could be embedded in coatings or paints, which would require a further study. Moreover, thanks to this project, future collaborations between Endures B.V. and Jose Palomo's laboratory are now being considered, in search of new solutions for the treatment of MIC.

In conclusion, this STSM has contributed to a better understanding of Microbiologically Influenced Corrosion and the behaviour of Sulphate Reducer Bacteria, Slime Forming Bacteria and Acid Producer Bacteria, as well as to the development of new mechanisms and compounds which help to prevent and mitigate it.