# Report on the outcomes of a Short-Term Scientific Mission[1]

**Action number: COST Action CA20130**

**Grantee name: Clara Ortega Nieto**

## Details of the STSM

Title: Control of marine biofilms with metal bionanohybrids

Start and end date: 15/10/2023 to 29/10/2023

## 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.

During this STSM, we have evaluated the capacity of 5 different metal-enzyme bionanohybrids developed in Prof. Jose M. Palomo's group (Institute of Catalysis, Spanish National Research Council) against biofilms of *Cobetia marina*, in Prof. Filipe Mergulhão laboratory (Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering of Porto).

In the first stage of this project, before this STSM, biofilms of *Cobetia marina* (strain DSM 4741/ATCC 25374) were developed for 7 weeks using glass coupons (1 cm x 1 cm) under dynamic conditions (25°C, 185 rpm and a shear rate of 40 $s^{-1}$), which simulates the marine environments, in Väätänen Nine-Salt Solution (VNSS) medium.

Then, biofilms were treated with 5 metal-enzyme bionanohybrids in a 500-ppm concentration for 6 hours under dynamic conditions (25 ºC, 185 rpm, 40 $s^{-1}$ shear rate) to evaluate their antimicrobial and anti-biofilm capacity, from the perspective of application of a disinfecting agent.

The bionanohybrids used for this experiment have been synthesized from different metallic salts using as scaffold the enzyme Lipase B from *Candida antarctica* (CAL-B) and were named with letters from A to E. The final exposure time differed from the initial working plan, where a 7-day exposition was planned from a different perspective. Two independent biological assays were made with two technical replicates per each one.

[1] This report is submitted by the grantee to the Action MC for approval and for claiming payment of the awarded grant. The Grant Awarding Coordinator coordinates the evaluation of this report on behalf of the Action MC and instructs the GH for payment of the Grant.

After that, biofilms analyses were performed through Optical Coherence Tomography (OCT), where it was measured the biofilm thickness and biovolume. For OCT imaging, it was used a Thorlabs Ganymede Spectral Domain Optical Coherence Tomography system with a central wavelength of 930 nm (Thorlabs GmbH, Dachau, Germany). Before the analysis, the coupons were washed once and the wells were filled with 3 mL of sodium chloride solution (8.5 g/L). For each coupon, 2D and 3D imaging were performed with a minimum of three points of view, to ensure the accuracy and reliability of the obtained results, following a method developed by Prof. Filipe Mergulhão's group.

The bacterial population was evaluated by counting the Colony Forming Units (CFUs). In addition, samples were stored to perform total cell analyses by flow cytometry if it would be possible.

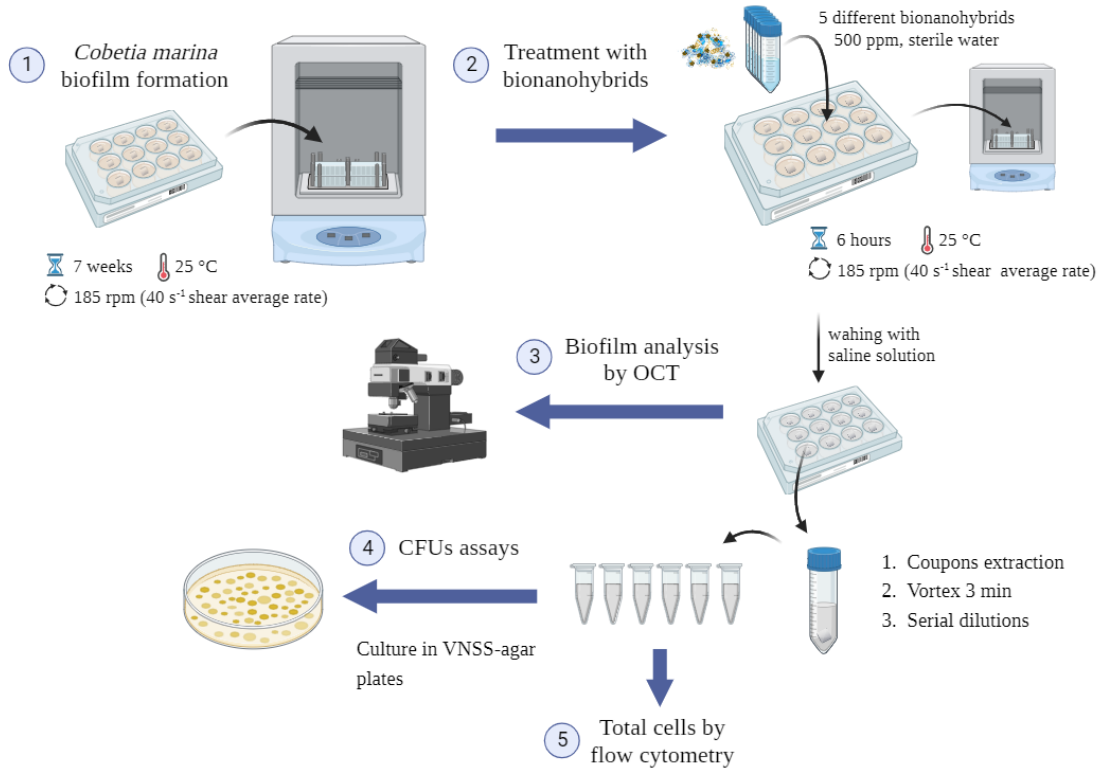


*Figure 1. Schematic illustration of the lab procedure*

To determine bacterial viability in the biofilms, each coupon was immersed in 2 mL of sterile 8.5 mg/mL NaCl and vortexed for 3 min to detach the bacteria from the coupon surface after the OCT imaging. Then, serial dilutions were performed (from -1 to -6) and 10 µL of the cell suspension of selected dilutions was cultured on VNSS agar plates in triplicate and incubated at 25 °C for 16 to 56 hours. After that, they were stored in the fridge and the colonies were counted to obtain the final bacterial concentration in CFUs/cm$^2$.

After the experimental work, data analysis was performed to obtain the results.

## 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.

Thanks to this STSM, bionanohybrids have been evaluated against *Cobetia marina* biofilms using different techniques. Through OCT imaging we have obtained 2D and 3D images of the biofilm architecture, which have been used to analyse structural parameters, as biovolume or thickness (Figure 2 and 3). However, the particle deposition of the hybrids on the coupons caused interferences, so the results may be taken with caution, as we can see in Figure 2.

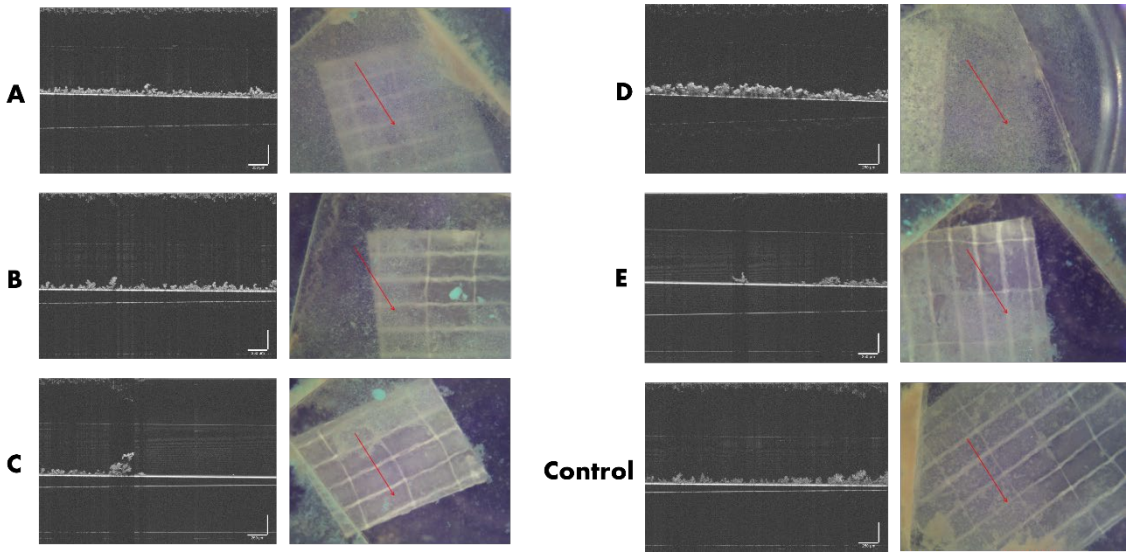


*Figure 2. 2D representative OCT images from Assay 1 obtained for the 5 bionanohybrids (A to E) and a control.*

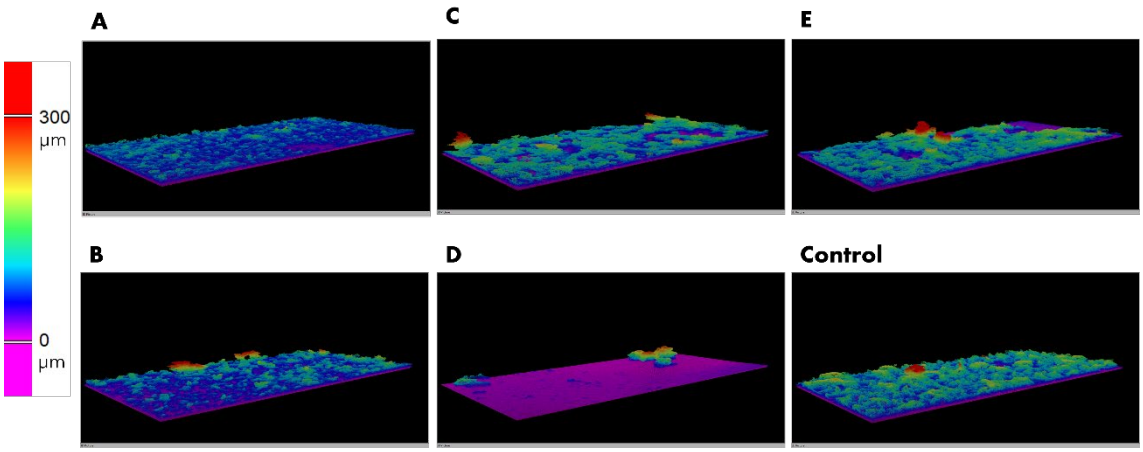


*Figure 3. 3D representative images from Assay 1 obtained for the 5 bionanohybrids (A to E) and a control.*

In thickness measurements, there is a slight inhibition compared to the control, except with the hybrids B and E, where thickness grows (possibly due to hybrid deposition on the surface, as seen in the pictures), as is shown in Figure 4. Even so, in the images taken of the biofilm, very bald areas can be seen in the coupon (Figure 3, D), together with areas where there was not much biofilm but hybrid particles.

In the biovolume measurements, we observed slight inhibition compared to the control, except again for hybrids A and E, which had higher values.

Thus, the results obtained in the analysis of biofilm thickness and biovolume through the OCT imaging were not as expected, so a change in the strategy and experimental procedure is being considered for further next steps.

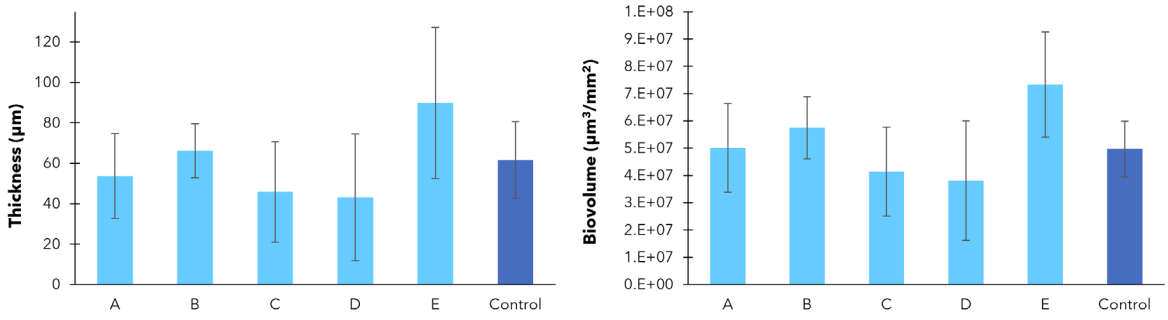


*Figure 4. Thickness and biovolume measures of C. marina biofilm before (biofilm 7 week) and after the treatment with the bionanohybrids (A to E and control). Mean values and SD from two biological assays with two technical replicates each are represented.*

However, regarding the CFUs results, it was found lower CFUs per $cm^2$ in the samples treated with the hybrids compared to the control, with a 2-log decrease with all the compounds tested. In addition, the final values were similar between all the bionanohybrids, ranging from $2.95x10^6$ to $8.03x10^6$ CFUs/$cm^2$.

Thus, the principal objectives of this STSM have been accomplished. We have shared the knowledge between two different groups about the development of new antimicrobial materials and their use and study against marine biofilms. In addition, thanks to this STSM we have taken a further step in the search for new materials that can be useful against MIC, with the aim of finding new and sustainable solutions, which is in line with the objectives of WG 4.

Furthermore, as a result of these experiments, new action strategies are being studied, looking for the optimal way to use and study the anti-biofilm effect of the bionanohybrids on different surfaces. Further collaboration between the two research groups involved is also being planned in view of the good antimicrobial results obtained.