

ENVIRONMENTALLY-INDUCED CHANGES OF COMMERCIAL CARBON

NANOTUBES IN AQUEOUS SUSPENSIONS.

ADAPTIVE BEHAVIOR OF BACTERIA IN BIOFILMS

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SUPPORTING INFORMATION

1. Experimental procedures to obtain the different MWCNT samples.

MWCNT samples mentioned in Table 1 of the main manuscript.

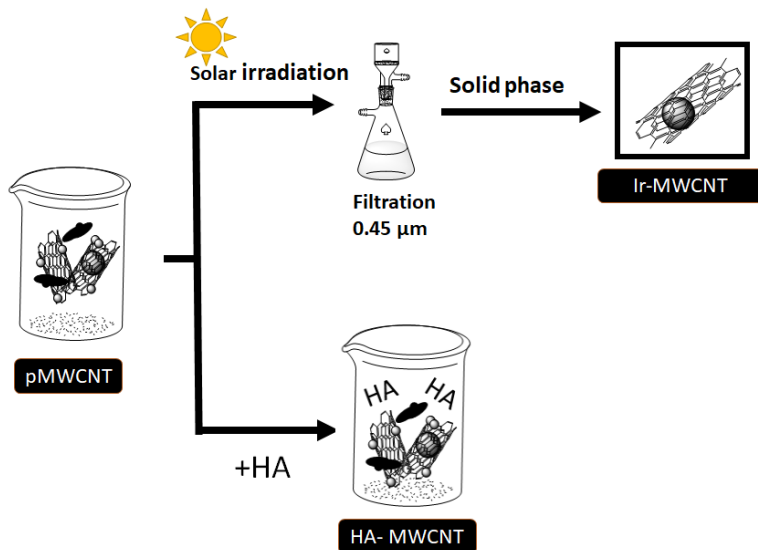


Figure S1. Experimental procedure to obtain Ir-MWCNT and HA-MWCNT mentioned in Table 1.

Figure S1 depicts the different steps of the experimental processes that lead to Ir-MWCNT and HA-MWCNT. The presence of catalytic metals used in the synthesis of the commercial nanotubes is represented with different shapes included in pMWCNT representation. They are absent in Ir-MWCNT to indicate that many of them were released during irradiation.

2. Statistical analysis of the size of bacteria growing in biofilms after being exposed to different types of MWCNT

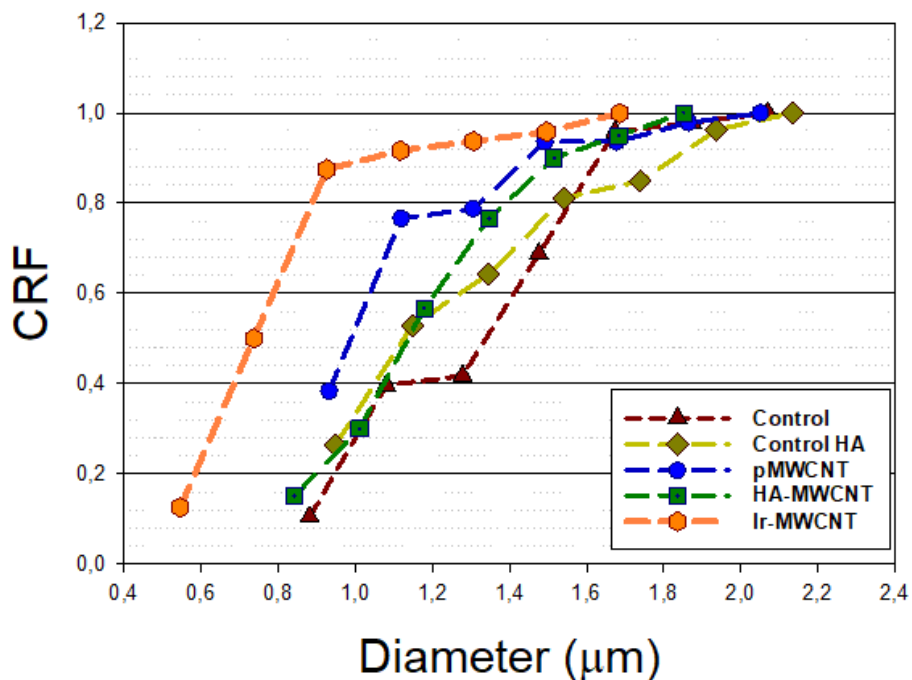


Figure S2. Cumulative relative frequency (CRF) of the size of bacteria after being exposed to different types of MWCNT. Distinct conditions of MWCNT and their Acronyms are indicated in Table 1 of the main document.

Statistical analysis of the distribution of the lengths of the bacteria growing in biofilms made using SEM microscopy shows (cumulative relative frequency, Figure S2) that for the non-Gaussian distributions depicted in the histograms of Figure 5, the median values can be ordered according to the following sequence: Control 1.30 μm > Control with HA (control-HA) 1.20 μm > HA-MWCNT 1.18 μm > pMWCNT 0.96 μm > Ir-MWCNT 0.74 μm. Higher median value for HA-MWCNT than that of pMWCNT are related to the shielding effect of HA.

3. Epifluorescence microscopy of glasses with biofilms stained with acridine orange

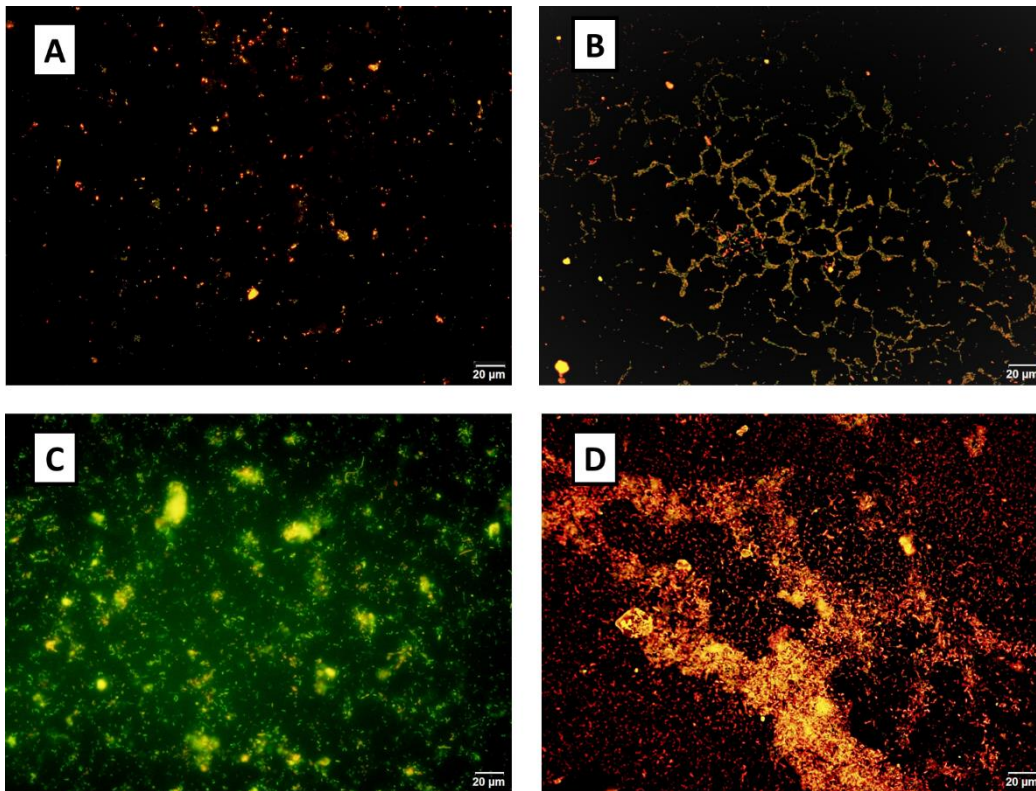


Figure S3. Epifluorescence micrographs of glasses with biofilms stained with Acridine Orange for A) control, B) pMWCNT, C) HA-MWCNT, D) Ir-MWCNT.

Epifluorescence image of the control (Figure S3 A) without the addition of nanotubes shows low bacterial adhesion in line with Figure 5 B of the main manuscript. The low adhesion is associated to the diluted nutrient broth used to emulate environmental conditions. Higher bacterial adhesion can be seen for HA-MWCNT assay. Green environments in Figure S3 C account for the healthy condition of the biofilm while orange regions suggest more toxic environment in Figure S3 D. A comparative analysis of the areas covered by the biofilms is made in the main manuscript.

4. Effect of MWCNT on biofilms after HA and Irradiation treatments (Ir-HA-MWCNT)

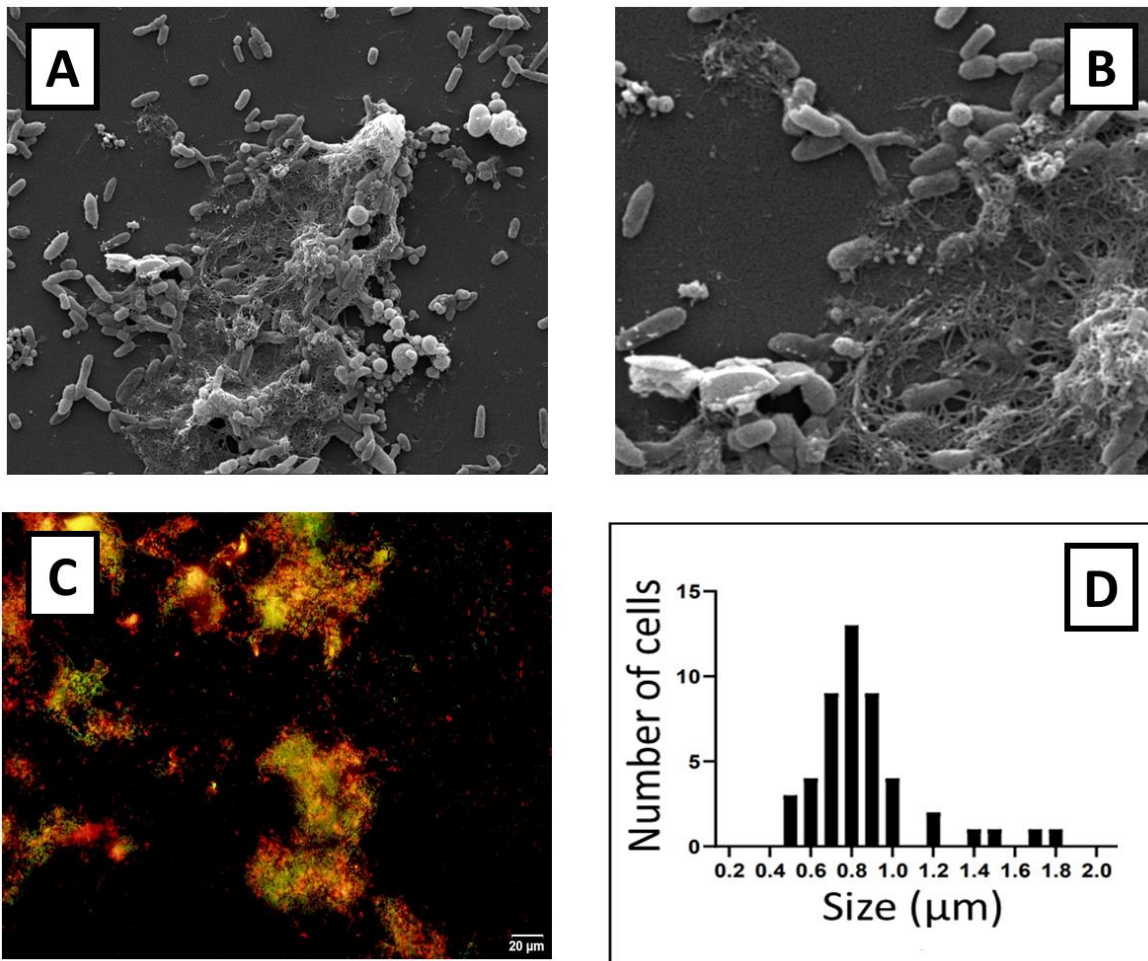


Figure S4. (A) SEM distribution of attached bacteria exposed to Ir-HA-MWCNT. (B) Detail of (S4 A), (C) epifluorescence microscopy image, (D) Distribution of bacterial sizes

Figure S4 A shows a SEM image revealing the overproduction of EPS related to the control (Figure 5 B of the main manuscript) when the Ir-MWCNT are exposed to HA (Ir-HA-MWCNT) and then added to the bacterial culture. An EPS strand-net (Figure S4 B) notoriously more compact than for Ir-MWCNT (Figure 5 E of the main manuscript) is formed. Epifluorescence microscopy of Ir-HA-MWCNT (Figure S4 C) shows green areas, absent in case of Figure S3 D (Ir-MWCNT). Bacterial size distribution of Ir-HA-MWCNT is also depicted on the right side (Figure S4 D) revealing a higher number of longer bacteria with a median value (0.81 μm) higher than that of Ir-MWCNT (0.74 μm).