

Effect of non-phosphorus corrosion inhibitors on biofilm pore structure and mechanical properties

Supporting Information

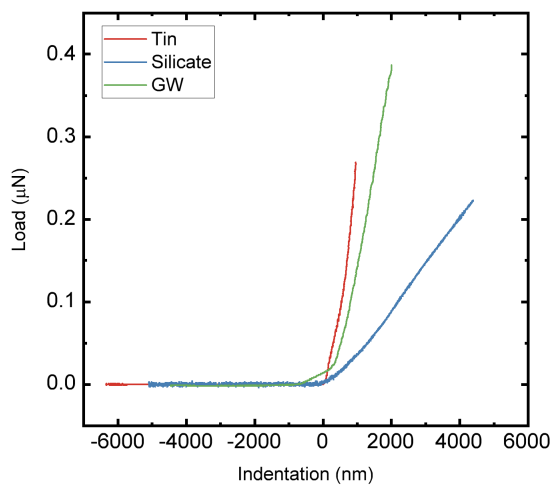
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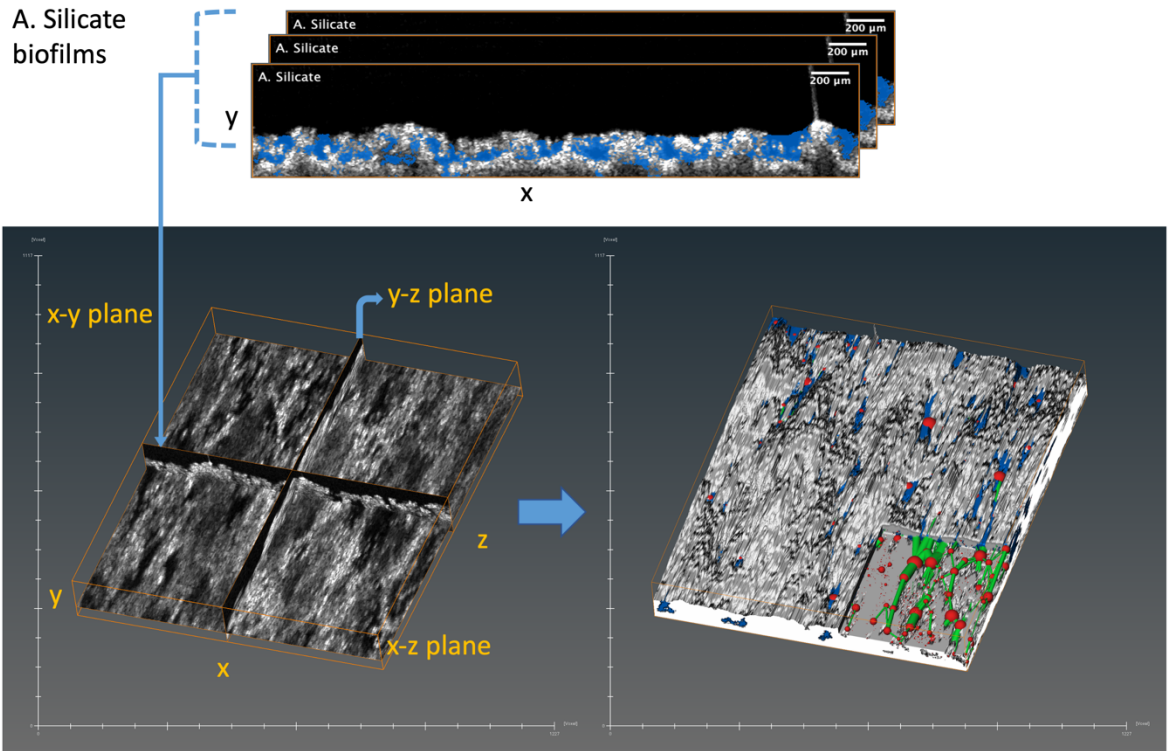
23 1. Bacteria presence in these three biofilms

24 The presence of biofilms in this study was confirmed by measuring the 16S genes in the
25 biofilm samples. We scraped and extracted DNA from silicate, tin, and groundwater biofilms
26 using FastDNA Spin Kit For Soil (MP Biomedicals). We determined the presence and absence
27 of bacteria by quantitative polymerase chain reaction (qPCR) using a primer set (341F:
28 CCTACGGGAGGCAGCAG; 518R: ATTACCGCGGCTGCTGG) amplifying the V3 region of
29 the 16S rRNA gene.¹ A reaction volume of 15 μ L containing 400 mM of forward and reverse
30 primers, 7.5 μ L of Applied BiosystemsTM PowerUpTM SYBRTM Green Master Mix (2x), 4.3 μ L
31 of nuclease-free water, and 2 μ L of DNA templates was prepared for each qPCR sample. The
32 DNA samples were serial diluted from 10 to 1000 times to avoid qPCR inhibition introduced
33 from EPS components. Triplicates were used in qPCR. The DNA was denatured at 95 °C for 10
34 minutes followed by 40 cycles of 15 seconds at 95 °C and 1 minute at 60 °C. Nuclease-free water
35 was used as the negative control. The corresponding cycle number (Ct), which was above 29,
36 served as the threshold of the presence of bacterial genomes in the DNA samples. Because lower
37 Ct numbers in the range of 19-23 were detected in the DNA extracted from the coupons (10 to
38 1000 times dilutions) compared to the negative control, biofilms have covered the PVC coupons
39 after at least 6 months in the CDC reactors fed by groundwater amended with different corrosion
40 inhibitors.

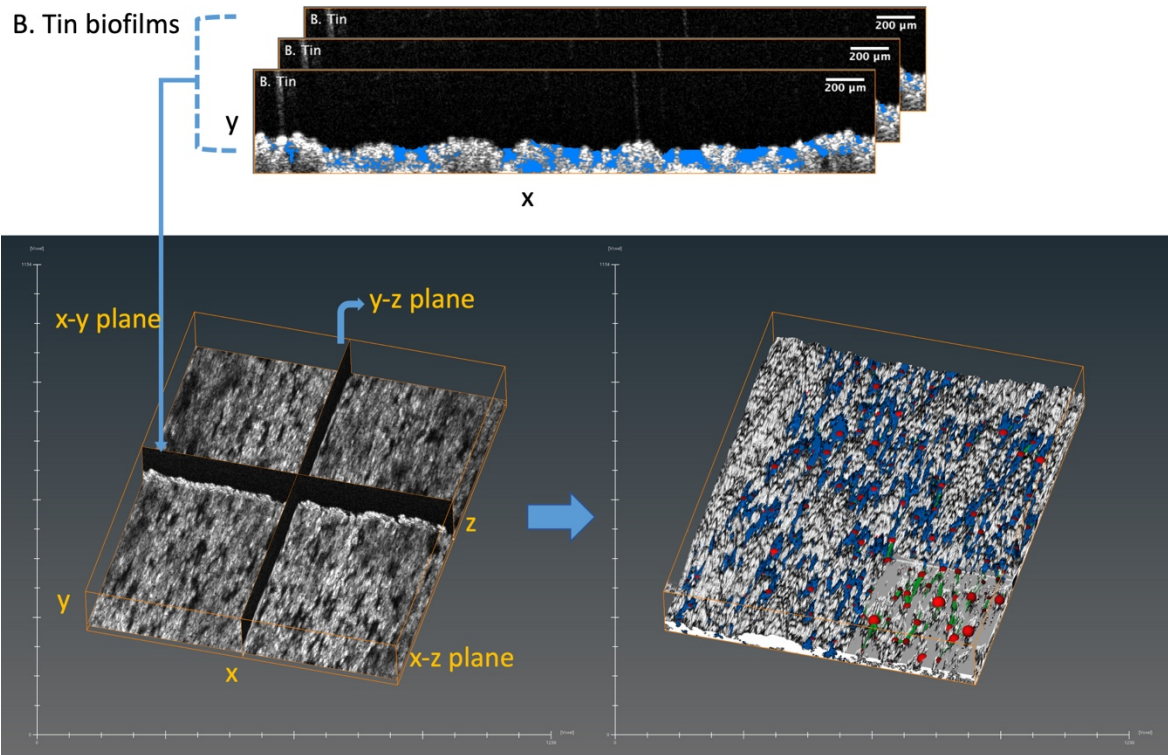
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43 **Figure S1:** A representative force (μN) vs. indentation (nm) of the tip during indentation to the
44 silicate biofilms (blue), tin biofilms (red), and groundwater biofilms (green).
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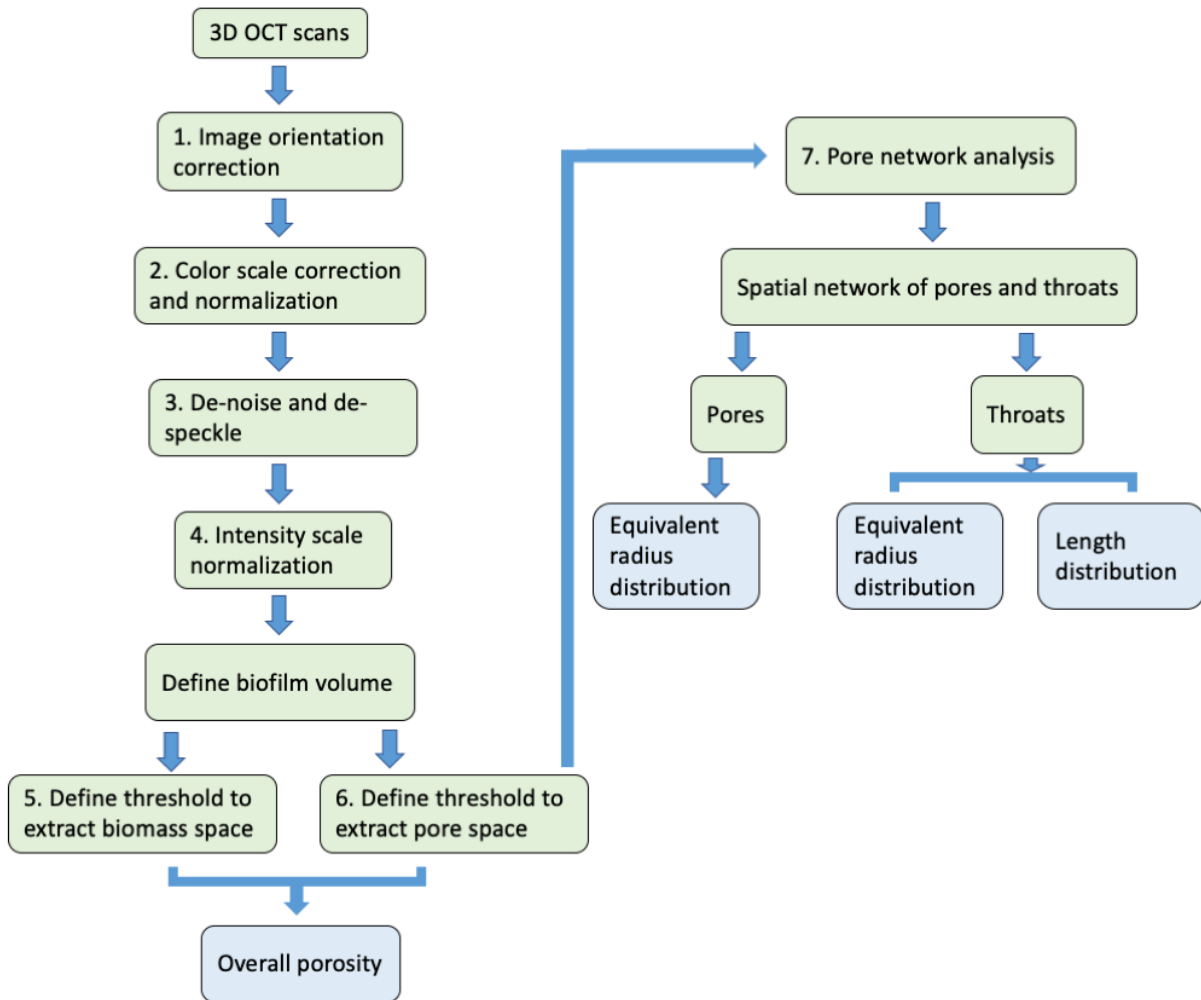
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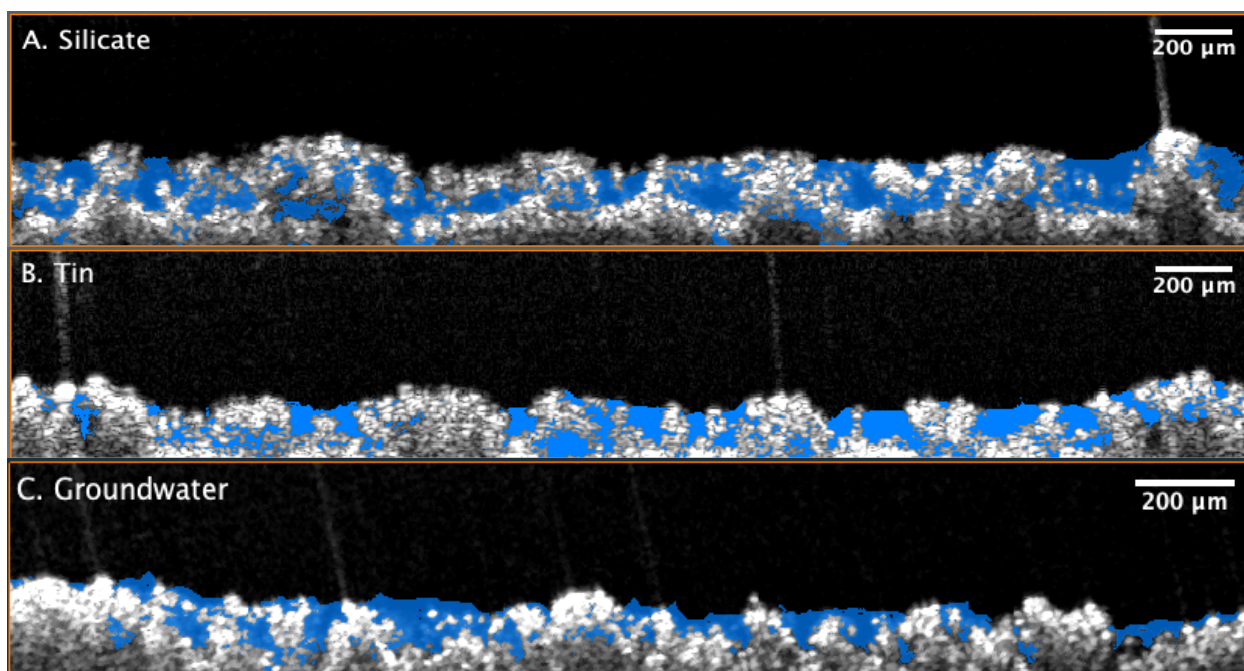
48 **Figure S2.** Image (x-y plane) stack obtained from OCT imaging scanning was shown in the top
 49 section. The ortho-slices (images orthogonal to the xy, yz, and xz plane) of a three-dimensional
 50 image stack were shown in the bottom left. Non-zero intensity pixels (white) represented
 51 biomass and zero intensity pixels (black) represented pore space. Orange lines marked the
 52 bounding boundary of the image stack. The representative of the 3D reconstruction of silicate

53 (A) and tin (B) biofilms were shown in bottom right. Blue marked the pore space; white marked
 54 the biomass. The pore space was characterized as a network constructed by red spherical pores
 55 connected by green cylindrical throats. The pore network was not drawn to scale. The upper 100
 56 μm from the maximum biofilm thickness in the right corner of the 3D reconstruction was
 57 removed to show the pore network.
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 62 **Figure S3.** Flow diagram of the image processing steps and the pore network analysis. Green
 63 boxes indicated the image processing procedures and blue boxes indicated the output results.
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Figure S4. Representative OCT images of A) silicate, B) tin, and C) groundwater biofilms. Biofilms were identified as white while pore space was identified as blue.

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74 **References**

- 75 1. Shen, Y.; Huang, C.; Lin, J.; Wu, W.; Ashbolt, N. J.; Liu, W.-T.; Nguyen, T. H.,
76 Effect of disinfectant exposure on *Legionella pneumophila* associated with simulated drinking
77 water biofilms: release, inactivation, and infectivity. *Environmental Science & Technology* **2017**.
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