SUPPLEMENTARY APPENDIX

Supplement to: **Multispecies colonisation and surface erosion on A106 GB industry-finished steel used in heat exchangers**

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Supplemental data

Stainless steel mesh that was inserted in the cooling tower coupon rack

Figure S1 Stainless steel mesh (white arrow) removed from the coupon rack (indicated with a red arrow).

Figure S2 Overview of the coupon rack near the cooling tower basin.

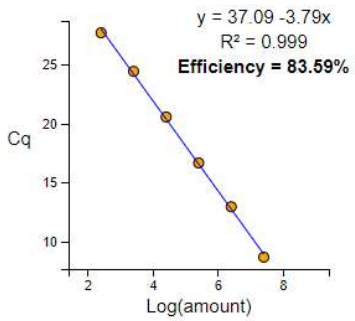

Figure S3 Standard curve with the 7 log dilutions (0.1 ng to 0.1 fg) and all the samples were calculated based on this curve.

The PCR efficiency was calculated based on the below formula:

$$E = \frac{10 - 1}{slope - 1}$$

The percentage efficiency was calculated based on the below formula:

$$Percentage\ efficiency = (E - 1) \times 100$$

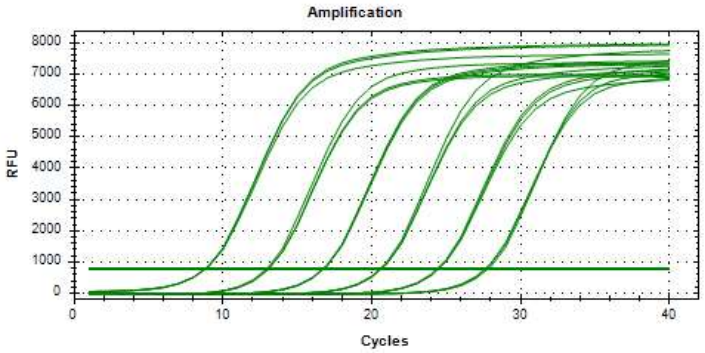

Figure S4 Amplification curves of standards.

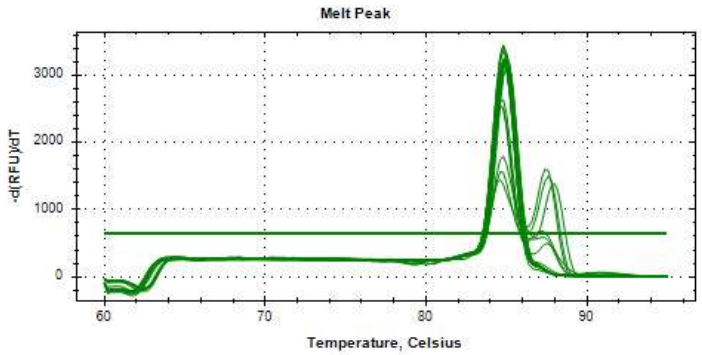

Figure S5 Melt peak of standards.

Table S1 Quantification of bacterial levels.

Sample[(a)]	Cq	Average Cq	Absolute copies
Day 3 smooth	18.25	18.34	88524.41
Day 3 smooth	18.43		
Day 3 smooth	18.34		
Day 3 rough	25.77	25.62	1062.1
Day 3 rough	25.54		
Day 3 rough	25.55		
Day 6 smooth	25.18	25.20	1368.40
Day 6 smooth	25.23		
Day 6 smooth	25.20		
Day 6 rough	24.26	24.27	2412.29
Day 6 rough	24.28		
Day 6 rough	24.27		
Day 13 smooth	25.09	24.91	1633.69
Day 13 smooth	25.10		
Day 13 smooth	24.54		
Day 13 rough	22.25	22.27	8127.98
Day 13 rough	22.22		
Day 13 rough	22.35		

(a) *Three replicates were run for each DNA sample to obtain the average Cq.*

The equation below was used to calculate the absolute copy number:

$$Genome\ copy\ \# = \frac{DNA}{g\ to\ bp\ constant\ \times\ genome\ size}$$

Where 0.1 ng of DNA was used, with a genome size of 3505 bp and g to bp constant of 1.096×10^{-21} g.

Plots on the dependance of bacterial adhesion extent as a function of roughness.

In light of the study the initial and selective attachment of a bacterial species was influenced by surface roughness. Middle and late colonising bacteria attach to the already present bacteria and biofilm. In the below plots (Figure S6 and S7), the roughness of the biofilm increases, and the bacterial attachment may be observed to be higher.

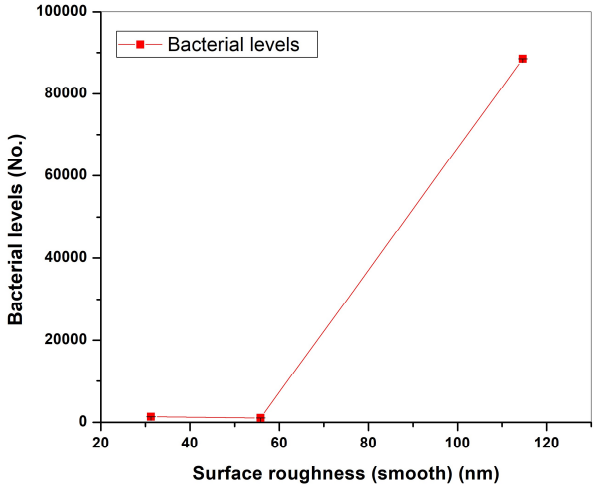

Figure S6 Bacterial levels on surface roughness on the smooth surface.

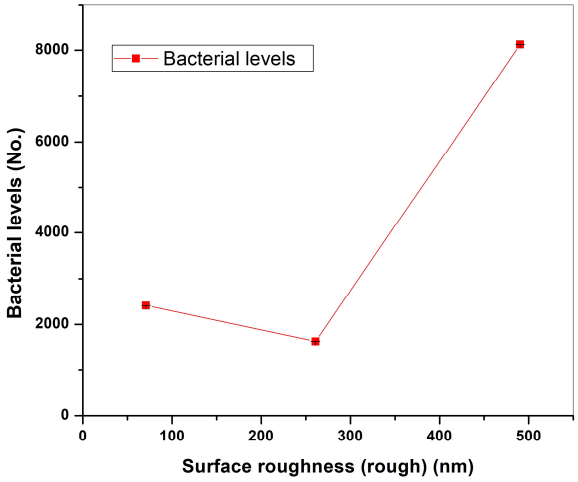

Figure S7 Bacterial levels on surface roughness on the rough surface.

Spatial distribution on the carbon steel surface

Figure S8 An example of the carbon steel coupons removed from the bacterial media on day 3, revealing the spatial arrangement of the biofilm on the surface.