

## **Impact of the air-liquid-material (ALM) interface on the formation and resistance of biofilms on different surfaces**

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Biofilms are ubiquitous in nature and can form on both biotic and abiotic surfaces, posing a significant threat to human health and the economy. While the formation of biofilms on fully submerged surfaces has been extensively studied, the impact of the air-liquid-material (ALM) interface on partially submerged surfaces has only received limited attention. In particular, the role of the ALM interface in the formation and resistance of biofilms by foodborne pathogens and non-pathogenic bacteria has not been very well characterized.

In this study, we investigated the impact of four strains (*Pseudomonas fluorescens* [Pf1], *Escherichia coli* [Ec-SS2], *Bacillus cereus* [Bc-98/4] and *B. subtilis* [Bs-PY79]) and four surfaces (2R and 2B finish stainless steel, polypropylene [PP] and glass) on the A-L-M (air-liquid-material) biofilm formation and resistance to cleaning to standard clean-in-place (CIP) process. A great difference in biofilm amount was observed between the strains. For instance, Bs-PY79 failed to form biofilm, while other strains formed biofilm in the range of 4.7 and 7.4 log CFU cm<sup>-2</sup>. For Ec-SS2 and Bc-98/4, PP surfaces held significantly less biofilm while no difference between materials in the case of Pf1 was observed. Upon subjecting biofilm to the CIP process (NaOH 0.5% at 60 °C), cultivable cells were only detected for Bc-98/4 biofilms (growth on agar), while biofilms were also still visible on coupons contaminated with Pf1. Furthermore, most residual biofilms after cleaning appeared orange by epifluorescence microscopy after staining with orange acridine suggesting the presence of many viable but non-culturable cells within the residual biofilms. Lastly, Bc-98/4 biofilms formed on stainless steel 2R were more resistant to cleaning than on PP and glass. These findings highlight the importance of considering the ALM interface in controlling biofilm formation and resistance, particularly in the food industry. Our results suggest that the susceptibility of surfaces to biofilm formation varies depending on the bacterial strain and surface material and that CIP processes may not be effective at completely removing biofilms from certain surfaces.