Biomimetic Surfaces as a Potential Strategy to Mitigate Biofouling in the Food Industry

Fábio M. Carvalho[1,2], Luciana C. Gomes[1,2], Marta Lima[1,2], Rita Teixeira-Santos[1,2], Ana Azevedo[1,2], Mohsin Amin[3], Mette Burmølle[4], Jelmer Sjollema[5], Filipe J. Mergulhão[1,2] and Kathryn A. Whitehead[3]

[1]LEPABE-Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal.

[2]ALiCE - Associate Laboratory in Chemical Engineering, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal.

[3]Microbiology at Interfaces, Faculty of Science and Engineering, Manchester Metropolitan University, Manchester M1 5GD, United Kingdom.

[4]Department of Microbiology, University of Copenhagen, Copenhagen, Denmark.

[5]Department of Biomedical Engineering, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands.

Presenter's e-mail address: up201502963@edu.fe.up.pt

Background: Contamination of food contact surfaces is a critical public health concern with economic impact. As standard hygiene procedures are constrained by the high resistance of foodborne pathogens, new technologies capable of restricting bacterial proliferation are imperative. Recent interest has focused on the development of antifouling surfaces with self-cleaning and superhydrophobic characteristics based on plants with different leaf topographies and physicochemical properties. This study aimed to (1) produce and characterize biomimetic surfaces that replicate the topographic features of vegetable leaves (White cabbage, WC; Tenderheart, TH; Cauliflower, CF; and Leek, L), and (2) evaluate their effectiveness in counteracting the earlier stages of biofilm formation (attachment, adhesion, and retention). Additionally, since the accumulation of organic matter on surfaces may change their physicochemical properties, the effect of a casein conditioning film on biofilm formation was evaluated and compared with that of non-conditioned surfaces.

Methods: Biomimetic surface replicates were synthesised by casting using dental wax. All surfaces were characterised regarding the water contact angle, surface hydrophobicity, roughness (by Optical Profilometry), morphology (by Scanning Electron Microscopy, SEM), and chemistry (by Fourier Transform Infrared Spectroscopy). For the attachment, adhesion and retention assays, culturable cells were enumerated by colony-forming units (CFU), and the surfaces were prepared for SEM imaging. Biofilm assays were performed in 12-well microtiter plates for 72 h under static conditions, and the resulting biofilms were analysed for

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the number of culturable cells by CFU count, biofilm thickness (by Optical Coherence Tomography) and biofilm architecture (by Confocal Laser Scanning Microscopy).

Results: Although there was a small loss in the resolution of the nano-surface features in biomimetic surfaces compared with the natural leaves, all types of biomimetic surfaces were more efficient at reducing the number of bacteria bound to the surface. *E. coli* attachment on biomimetic surfaces was influenced by both surface hydrophobicity and roughness, where the WC (roughest and most hydrophobic surface) showed the lowest cell numbers (approximately 1 Log CFU/cm^2 of reduction comparing with the natural leaf). Following the retention assays, all biomimetic surfaces were more efficient than natural leaves, exhibiting reductions of about 2 Log CFU/cm^2, with biomimetic surfaces of WC, TH and L showing lower *E. coli* retention than the flat control surface. Moreover, a significant decrease in the number of culturable cells was observed in *E. coli* biofilms formed on conditioned (up to 50% reduction) and non-conditioned biomimetic surfaces (up to 89% reduction) compared with the flat surface.

Conclusions: The results evidenced casting as a reliable technique to reproduce the structure of vegetable leaves, and these surfaces seem to be promising for preventing bacterial binding and mature biofilms.

Keywords: Biomimetic surfaces; Plant leaves; Biofouling; Conditioning film; Food industry.

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