Anti-biofilm coatings on stainless steel for food contact applications via plasma-polymerization

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Introduction

- The **microbial colonization of food contact tools, surfaces and equipment** in the form of **biofilms** may lead to the **cross contamination** of food products.
- **Listeriosis** is one of the most serious food-borne diseases, with an increasing trend of confirmed cases in the EU/EAA observed in recent years and a case **fatality of 15.6%.**
- **Conventional compounds** used for cleaning and disinfection in food industries do not eliminate bacterial biofilms completely and their use might imply **health and environmental risks**, and generate **bacterial resistance** or tolerance phenomena.
- **Coatings that modify the physico-chemical properties** of food-contact surfaces can **prevent microbial attachment** (first step for biofilm formation) without using biocidal agents, thus being able to produce **non-toxic surfaces with antibacterial effects**.
- **OBJECTIVE:** To reduce *Listeria monocytogenes* biofilm formation using a **coating** applied by **atmospheric pressure plasma-polymerization** on stainless steel (SS) plates.

Methods

- An Atmospheric-Pressure Plasma Jet (APPJ) system was used to coat AISI 316 SS plates (**Figure 1**). **The coatings comprised two parts** that were deposited using different precursors: **(1) a base coating** of (3-aminopropyl)triethoxysilane (APTES) and **(2) a functional coating** of tetraethyl orthosilicate (TEOS), a 0.3M solution of succinic acid (SuAc) or acrylic acid (AcAc).
- The uncoated SS and the coatings were characterized **chemically** (XPS) and **morphologically** (SEM and AFM) and their **wettability** was studied by measuring their water contact angle (WCA) (**Figures 2 and 3[a]**).
- To study the anti-biofilm effect of the coatings, biofilm formation by *L. monocytogenes* CECT911 was quantified by crystal violet (CV) staining after incubation in two conditions: 37 ºC/24 hours and 12 ºC/144 hours (**Figure 3[b]**). In all the cases, control plates without coating were included.
- The cellular hydrophobicity of the *L. monocytogenes* CECT911 strain was determined with the adhesion-to-hydrocarbon method in the two incubation conditions of this study: 37 ºC/24 hours and 12 ºC/144 hours (**Figure 4**).

Fig. 1. [a] Setup used for the plasma-polymerization process; [d] Scheme of plasma-polymerization process.

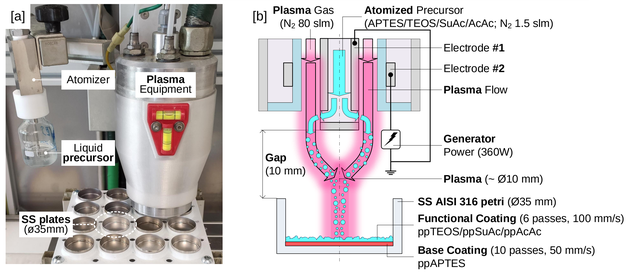


Fig. 2. Characterization of [a] the uncoated SS substrate, [b] the base coating of APTES, and the functional coatings [c] APTES-TEOS, [d] APTES-SuAc and [e] APTES-AcAc: [1] SEM images, [2] AFM images and average roughness (Ra), [3] C1s region of the XPS spectra deconvoluted for the quantification of polar oxygen-containing groups and [4] WCA measurements.

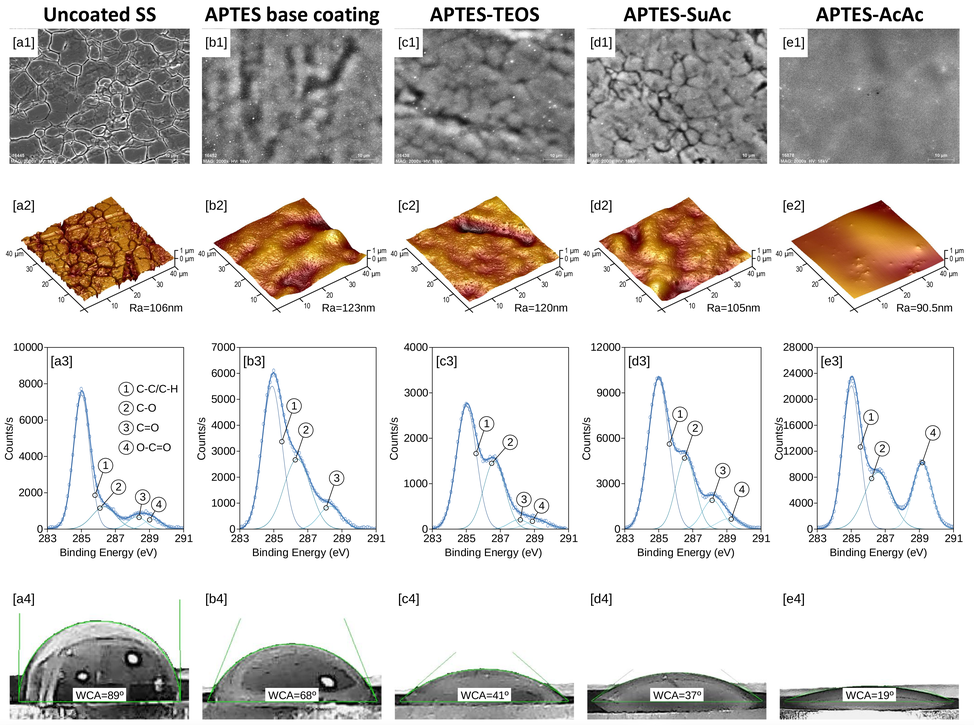


Fig. 3. [a] Concentration of O-C=O species in the C 1s region of the X-ray photoelectron spectra and water contact angles of the functional coatings; [b] Biofilm production on each coating, relative to that on the uncoated SS, in two incubation conditions.

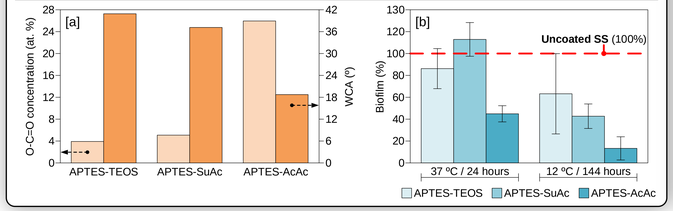


Fig. 4. Cellular hydrophobicity of *L. monocytogenes* CECT911 in two incubation conditions.

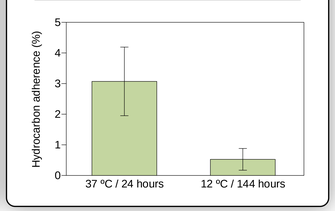
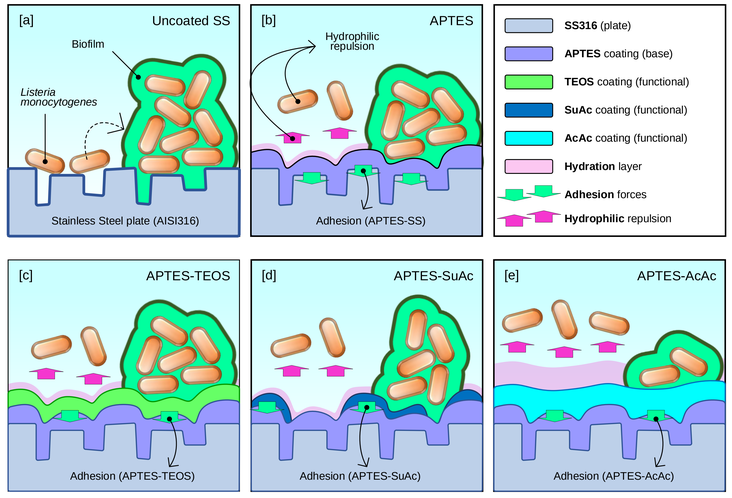


Fig. 5. Interaction between *L. monocytogenes* and [a] the uncoated SS plates, [b] the base coating of APTES and the functional coatings [c] APTES-TEOS, [d] APTES-SuAc and [e] APTES-AcAc, showing different degrees of cell repulsion according to the generation of hydration layers.



Conclusions

- The **hydrophilic character** of the coatings, that can be a result of the increased abundance of **oxygen-containing polar groups** (C-O, C=O and especially **O-C=O**) **(Figure 3)**, suggests that a **hydration layer** might have acted as a water barrier against bacterial cells and proteins **(Figure 5)**.
- The **reduction in the occurrence of grooves** of the SS substrate can **reduce the entrapment of bacterial cells** in zones with high cell-surface contact area.
- ***L. monocytogenes*** cells were less hydrophobic and seemed **less prone to adhere** to the studied surfaces at **12 ºC** than at 37 ºC, thus leading to **lower biofilm production**.
- The most promising coating was **APTES-AcAc**. It was the **most hydrophilic** and its surface was the smoothest and showed **no grooves**. **It reduced the formation of *L. monocytogenes* biofilm to 13.3%** and 44.9% relative to that on the uncoated SS after incubation at 12 ºC and 37 ºC, respectively.
- The increased effectiveness of the coatings at a relatively **low temperature (12 ºC)**, representative of the conditions prevailing during **food processing**, would facilitate their implementation in the food industry.
- Tests with **other microorganisms** are needed. Considering the fact that several pathogenic microorganisms usually coexist in food-processing environments, future work will characterize the effectivity of the coatings on **mixed-species biofilms** to validate the usefulness of this technology in realistic settings. Also, the **toxicity and durability** of the coatings will be evaluated.

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