Durability Study of the Anti-biofilm Capacity of Plasma-Polymerized Coatings on Stainless Steel for Food Contact Applications

PLASMA PROCESSING AND TECHNOLOGY INTERNATIONAL CONFERENCE
PLASMA TECH 2021
07 - 09 APRIL, 2021
PARIS - FRANCE

R. Múgica-Vidal[1,*], P. Fernández-Gómez[2], I. Muro-Fraguas[1], A. Sainz-García[1], M. González-Raurich[2], A. Álvarez-Ordóñez[2], M. Prieto[2], M. López[2], P. Toledano[3], M. López[3], Y. Sáenz[3], A. González-Marcos[1], F. Alba-Elías[1], E. Sainz-García[1]

*[1]Universidad de La Rioja (Logroño, **Spain**), [2]Universidad de León (León, **Spain**), [3]Centro de Investigación Biomédica de La Rioja (Logroño, **Spain**)*

[1] UNIVERSIDAD DE LA RIOJA
[2] universidad de león
[3] CIBIR Centro de Investigación Biomédica de La Rioja

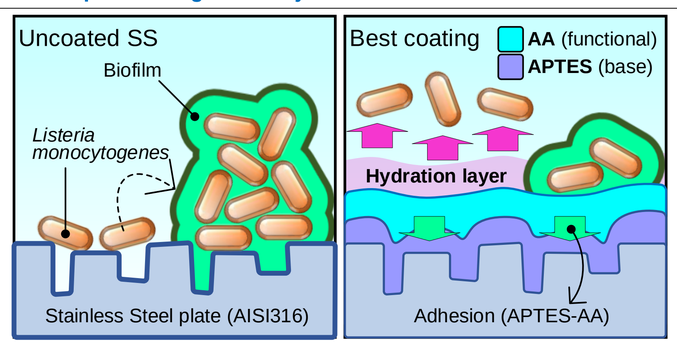
Introduction

Food industry problems

- Persistent microbial colonies in the form of biofilm attached to **food contact tools, surfaces and equipment** cause **cross-contamination** of food products.
- **Listeriosis** is a food-borne disease with a case **fatality of 15.6%.** It has shown an **increasing trend of confirmed cases** in the EU/EAA in recent years.
- **Conventional cleaning and disinfection compounds** do not eliminate bacterial biofilms completely, which can generate **bacterial resistance or tolerance** phenomena. **Intensive use** of these compouinds also poses **health and environmental risks.**

Previous work

P. Fernández-Gómez, I. Muro-Fraguas, R. Múgica-Vidal, E. Sainz-García, M. González-Raurich, M. Prieto, M. López, M. López, Y. Sáenz, A. González-Marcos, F. Alba-Elías, Development and characterization of anti-biofilm coatings applied by Non-Equilibrium Atmospheric Plasma on stainless steel, Food Res. Int. (2020) 109891. In press. https://doi.org/10.1016/j.foodres.2020.109891



- **Single-strain biofilm** production by *Listeria monocytogenes* was **reduced by 90%**.
- **Low roughness** and **strong hydrophilicity** reduce the initial atachment of *L. monocytogenes*.

Objectives

- To confirm the effectiveness of the best coating from previous work at **reducing multiple-strain biofim** production by *L. monocytogenes*, including **strains isolated from food industries**.
- To determine that the anti-biofilm effectiveness is **durable** when the surface of the coating is **cleaned with two commonly used disinfectants** of different chemical natures: (1) acid and (2) alkaline.

Methods

- AISI 316 SS plates (**Figure 1**) were coated using an Atmospheric-Pressure Plasma Jet (APPJ) system with **(1) a base coating** of (3-aminopropyl)triethoxysilane (APTES) and **(2) a functional coating** of acrylic acid (AA).
- The uncoated SS and the coatings were subjected to **5 cleaning cycles (Figure 2)** with solutions of **sodium hypochlorite (NaOCl, 0.05%)** and **peracetic acid ($C_2H_4O_3$, 0.5%)**.
- Uncoated and coated AISI 316 SS disks were also used for studying the **evolution of the wettabillity** of the surfaces by measuring their **water contact angle (WCA)** before cleaning and after each cleaning cycle **(Figure 3[a])**. Also, their **surface chemistry after the 5 cleaning cycles** was analyzed by X-Ray Photoelectron Spectroscopy (XPS) **(Figure 3[b])**.
- To study the anti-biofilm effect of the coatings as-deposited (without cleaning) and after 5 cleaning cycles, biofilm formation by a **three-strain cocktail** of *L. monocytogenes* (CECT911, ULE1264 and ULE1265) was quantified by crystal violet (CV) staining after incubation at 12 ºC for 6 days (**Figure 4**). In all the cases, control plates without coating were included.

Results & Discussion

- Although the cleaning solutions induced some degree of hydrophilicity on the uncoated SS, **for each solution the surface of coating AP10+AA6 was always more hydrophilic** (i.e., exhibited a lower WCA) than that of the uncoated SS. Also, after 5 cleaning cycles with either of the two cleaning solutions, the coating still showed **anti-biofilm effectiveness (biofilm production <100%)**. This suggest that **the coating preserved its effectiveness at preventing bacterial adhesion** to a certain degree during subsequent cleaning cycles.
- More remarkably, the coating always kept a **strong hydrophilic character (WCA < 20º)** when it was cleaned with **sodium hypochlorite (0.05%)**. Also, after 5 cleaning cycles with this solution, a **higher concentration of polar carbon-oxygen groups** was kept than after cleaning with peracetic acid (0.5%), and the **biofilm production on coating AP10+AA6 was still substantially lower (28%)** than on the uncoated SS. Therefore, an acceptable degree of anti-biofilm capacity was maintained, which suggest that **coating AP10+AA6 and the cleaning solution of sodium hypochlorite (0.05%) are compatible.**
- Since this study **resembled the conditions prevailing in food processing environments** by employing an incubation temperature of **12 ºC** and a bacterial cocktail containing **two *L. monocytogenes* strains from food industries**, the findings of this study are promising for enabling the **industrial implementation** of coating AP10+AA6.
- To better understand how the coating is affected by repeated cleaning, **morphological characterization** of the coating **after cleaning** (e.g., by SEM or AFM) will be necessary. Also, in order to ascertain the safety of the coating, its **toxicity** will be evaluated in future work.

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

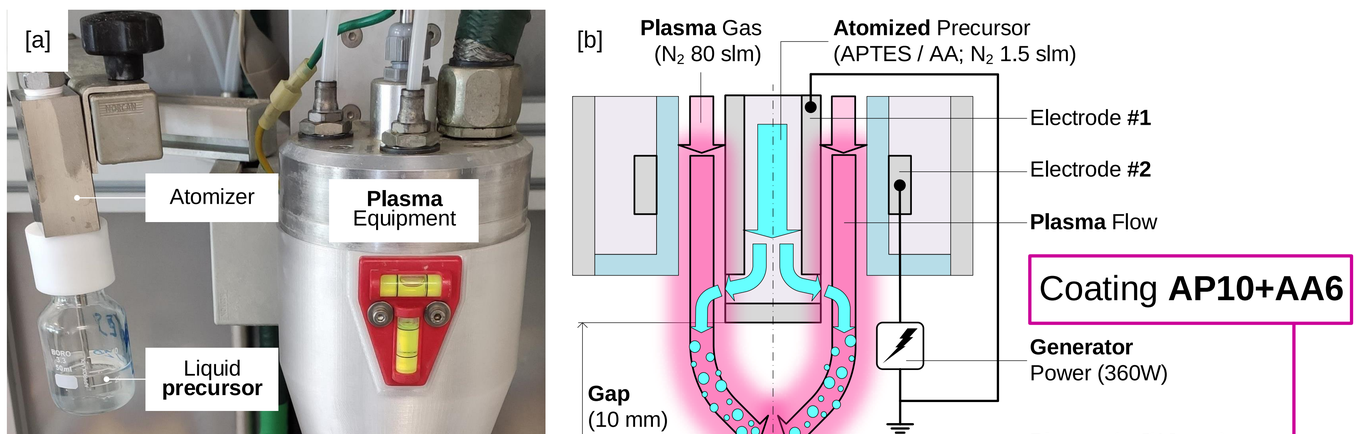


Fig. 2. Sequence that was followed for coating, cleaning and analyzing the SS disks and plates.

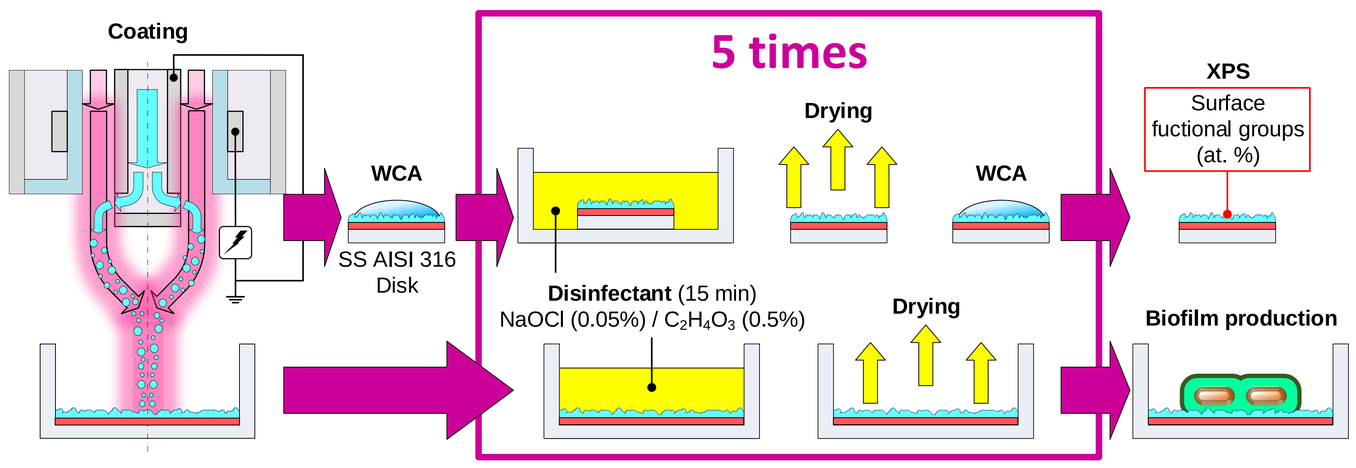


Fig. 3. [a] Evolution of the WCA of the uncoated SS and coating AP10+AA6 with 5 cleaning cycles using sodium hypochlorite (0.05%) and peracetic acid (0.5%). [b] WCA of coating AP10+AA6 and polar carbon-oxygen species cocentration (C-O, C=O and O-C=O) in the C1s region of its XPS spectrum.

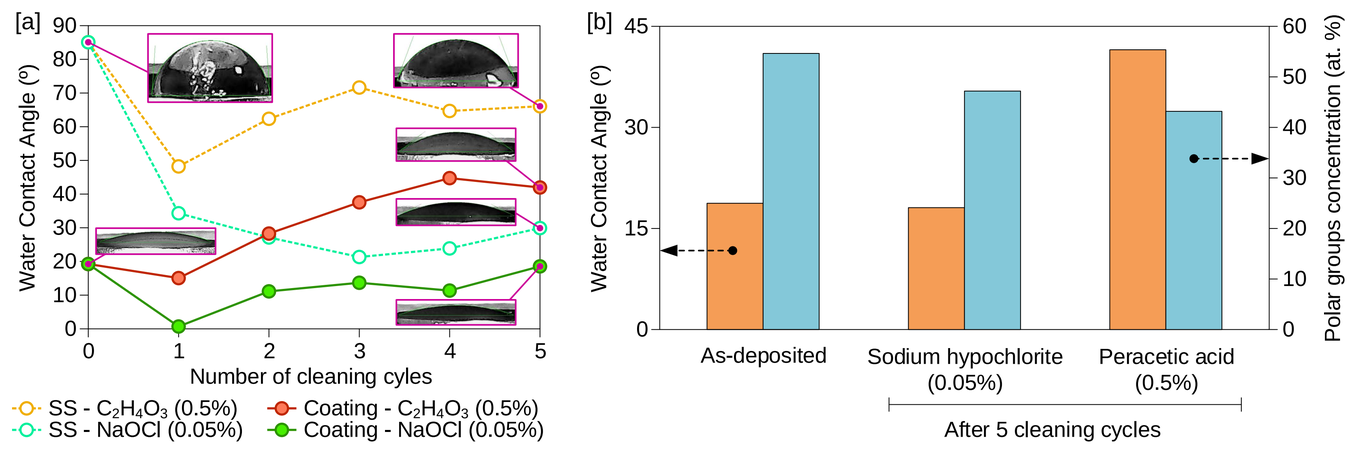
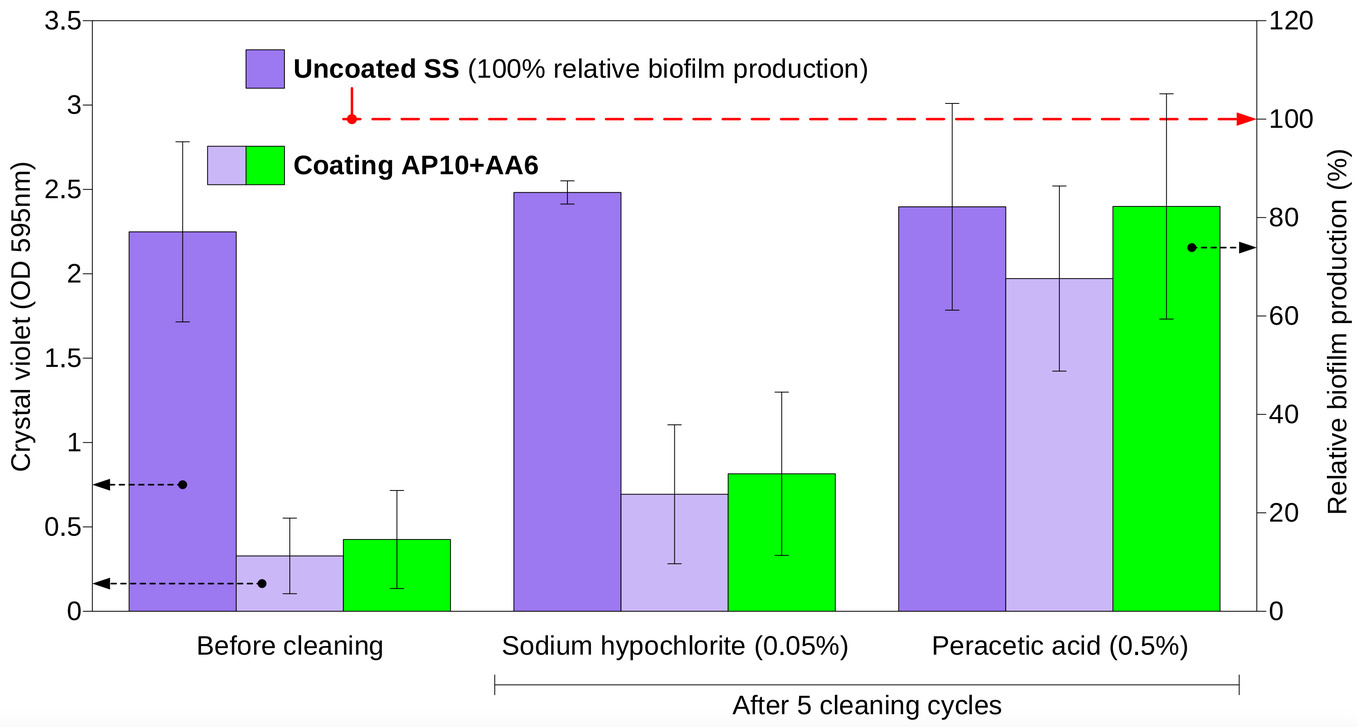


Fig. 4. Optical densities (595 nm) in the crystal violet assays and relative biofilm productions of the uncoated SS substrate and coating AP10+AA6.



Corresponding author: Dr. Fernando Alba-Elías. Department of Mechanical Engineering, La Rioja University, C/ San José de Calasanz, 31, 26004 Logroño, La Rioja (Spain). *(fernando.alba@unirioja.es)*

ACKNOWLEDGMENTS: This work was supported by Ministerio de Economía, Industria y Competitividad from Spain (MINECO) (project **AGL2017-82779-C2-R** "Programa Estatal de I+D+i Orientada a los Retos de la Sociedad") and co-funded by the European Regional Development Fund (FEDER) "A way to make Europe". XPS tests were conducted by the Advanced Microscopy Laboratory (LMA) of The Institute of Nanosciences of Aragón (INA), University of Zaragoza. The authors are thankful to the LMA-INA for the access to their equipment and their expertise. P. Fernández-Gómez is grateful to Junta de Castilla y León and the European Social Fund (ESF) for awarding her a pre-doctoral grant (BOCYL-D-15122017-4). The author I. Muro-Fraguas thanks the program of pre-doctoral contracts for the training of research staff funded by the University of La Rioja. The author E. Sainz-García, as postdoctoral researcher of the University of La Rioja, thanks the post-doctoral training program that is funded by the Plan Propio of the University of La Rioja.

Equipment provided by: mpg. molecular plasma group