Adhesion of *E. coli* on Commonly Used Kitchen Worktops: Wood, Granite, Melamine and Teflon

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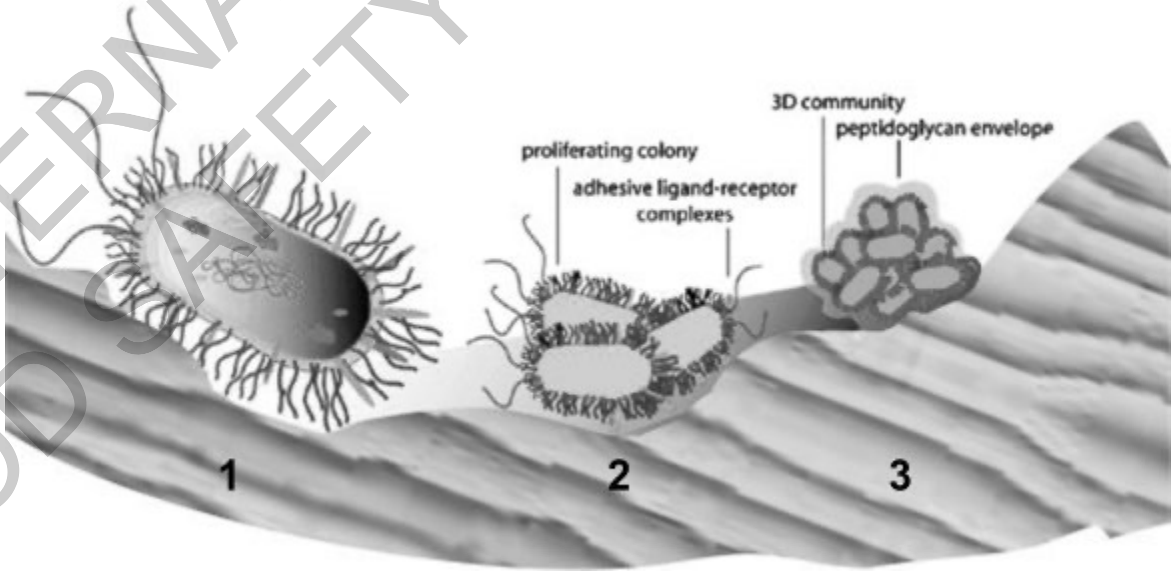
Content

- Introduction
- Material surface characterization
- Bacterial surface characterization
- Bacterial adhesion rate measurements
- Outlook

Aim: investigate bacterial adhesion on different kitchen material surfaces

Biofilms and food safety

Biofilms have been of considerable interest in the context of food hygiene and food safety. In nature and food systems, microorganisms get attracted to solid surface conditioned with nutrients, that are sufficient for their viability and growth.



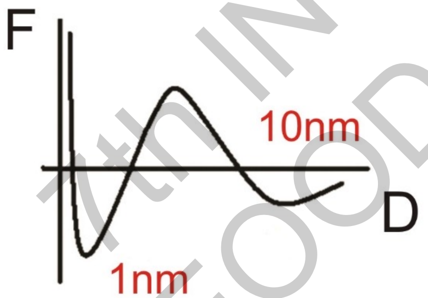
Biofilms and food safety

Contact material and bacterial surface properties play an important role in food safety and technology.

Biofilm has been defined as complex, three-dimensional functional consortia of adherent microorganisms, bound to, and growing at an interface and encased by an extracellular polymeric matrix (Mack 2007, Walker 2004, Kawarai 2009).

Biofilms and food safety

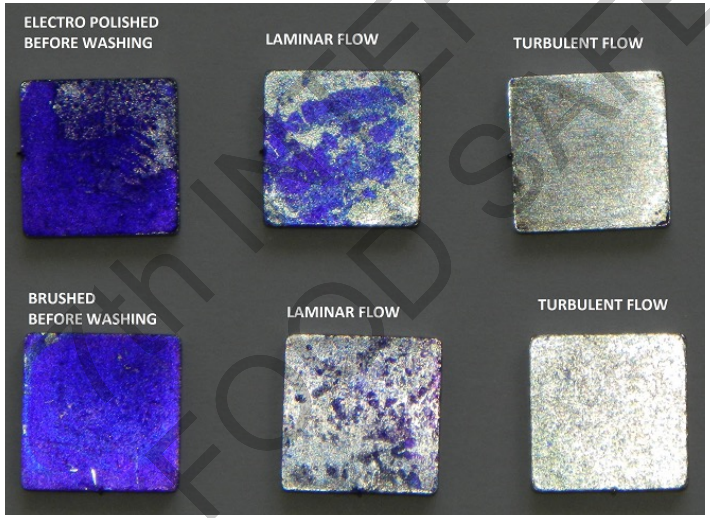
- In food industry the interaction between MO and food-contact surfaces is of fundamental importance regarding food safety issue.
- Microbial contamination of food-contact surfaces is an ongoing concern for the food industry. *Floors, walls and open working surfaces, such as benches, sinks, tables, conveyers, represent one of the principal sources of microbial contamination in food industry.*
- Under favorable conditions, bacterial cells can adhere, multiply and be addressed as a source of contamination.

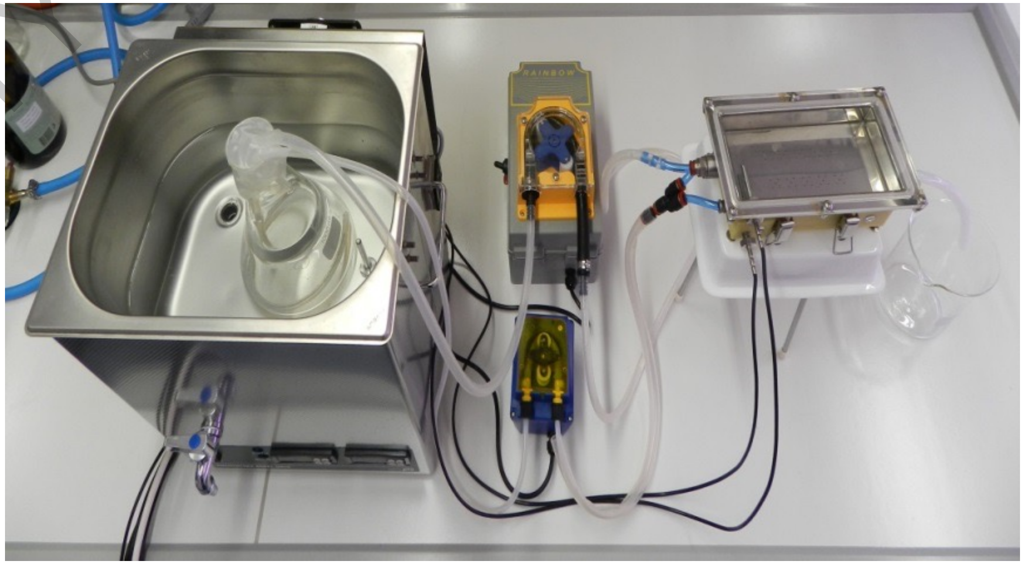


Biofilm identified with the Biofilm Detection Kit

Sanitation of food contact surfaces

Poor sanitation of food contact surfaces, equipment, and processing environments has been recognized as a contributing factor of food borne disease outbreaks, especially those involving *Listeria monocytogenes* and *Salmonella*.

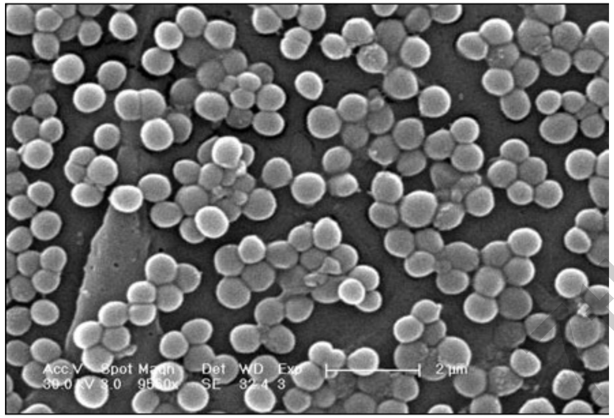




Factors affecting bacterial adhesion

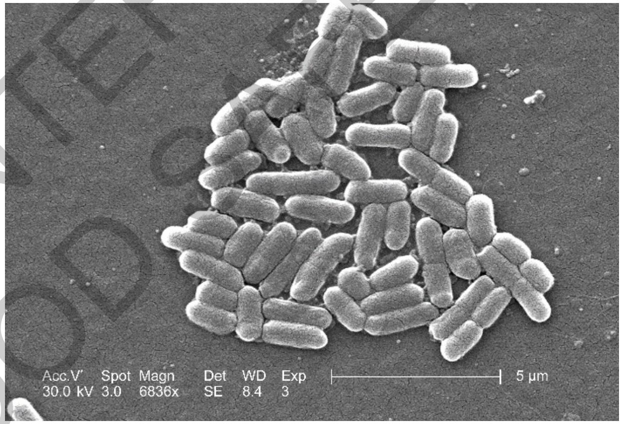
- Material surface characteristics (roughness, surface charge hydrophobicity)
- Bacteria surface characteristics (charge, hydrophobicity, surface specificity)

● *Staphylococcus aureus* (Gram positive)



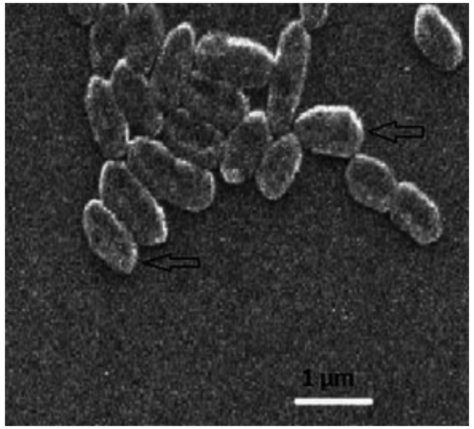
* Centers for Disease Control and Prevention

● *Escherichia coli* (Gram negative)



* https://www.ciriscience.org/ph_12-Escherichia-coli-O157H7-BandW

● *Pseudomonas aeruginosa* (Gram negative)

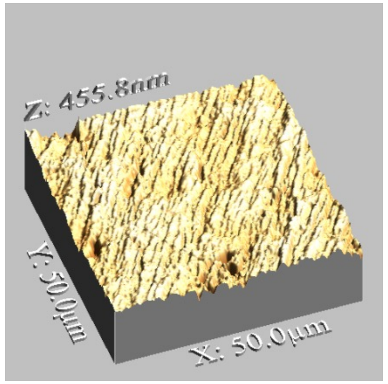


*R. K. Kunkalekar, M. M. Naik, S. K. Dubey, A. V. Salker; Journal of Chemical Technology & Biotechnology 05/2013; 88(5):873–877. DOI:10.1002/jctb.3915

Material-Stainless steel

- **Wood**
- **Granite**
- **Melamine**
- **Teflon**
- **Polietilen tereftalat (PET)**
- **Aluminium**
- **Silicone**
- **Glass**
- **Ceramics**
- **Stainless Steel**

- Materials were cut in pieces 10 × 10 mm.

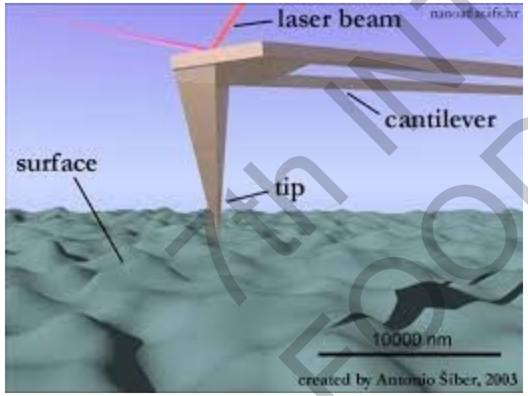


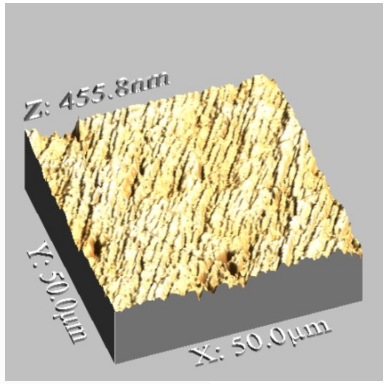
Surface roughness

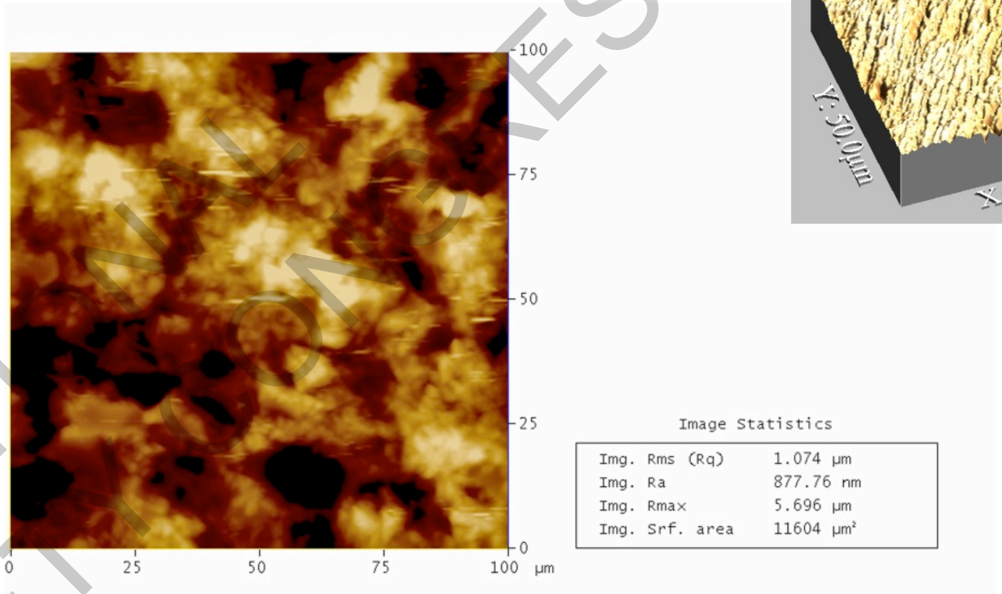
- characterization of surface topography

- AFM: VEECO Dimension 3100 AFM, contact mode
- mechanical profilometer:

  Form Talysurf Series 2 from Taylor Hobson Ltd.







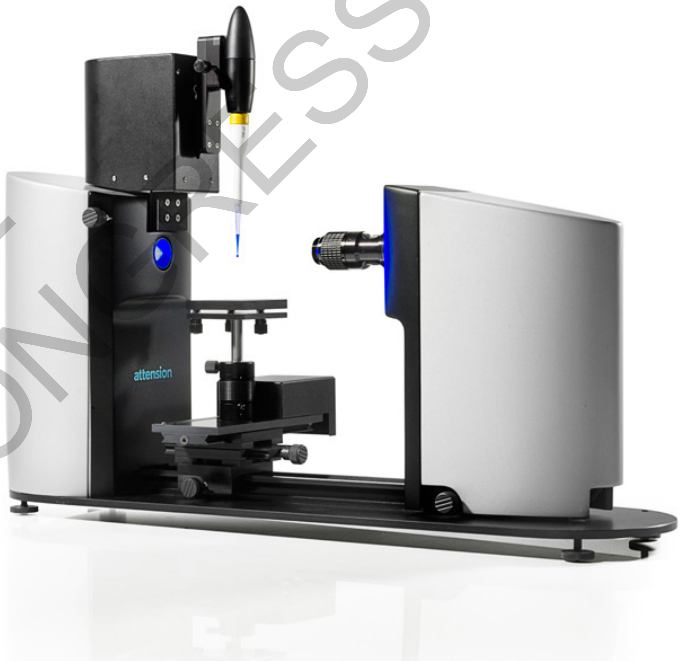
surface ground to gradation of P500

$$Ra = \frac{1}{M\,N}\sum_{i=1}^{M}\sum_{j=1}^{N}\left|z_{ij}\right|$$

z_{ij} elevation of the rough surface

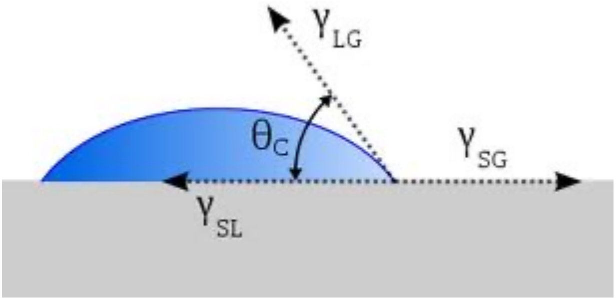
Contact angle

- contact angles between drop of water and substrate
- Theta Optical Tensiometer (Biolin, Attension, Finland)
- Using dispenser a small drop of water was placed on the substrate
- Using digital camera and light source at opposite directions of the drop an image of the profile of the drop is taken
- With dedicated software the angle between the substrate and the drop is measured from the image



$$\cos\theta_c^* = r\cos\theta_c$$

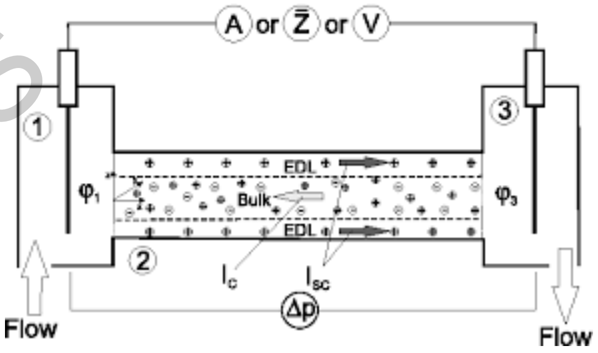
r ≥ 1, surface roughness decreases the contact angle



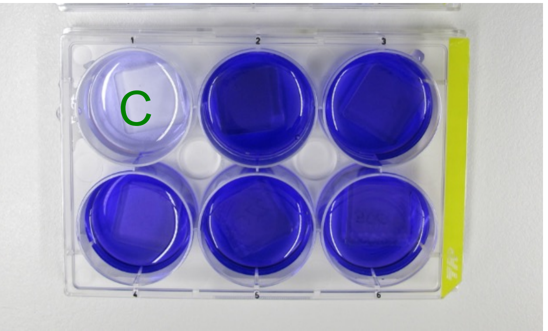
Streaming potential measurement

- The surface charge analysis was accomplished using an electro-kinetic analyzer (SurPASS, Anton Paar GmbH, Austria).
- The zeta potential was obtained in a 1 mM phosphate-buffered saline (PBS) solution via streaming potential measurements at room temperature.
- The zeta potential is related to the surface charge at a solid/liquid interface.

- $$\Delta U = \frac{\varepsilon \varepsilon_0 \zeta}{\eta k_L} \Delta p$$



Monitoring the adhesion



Staining

Incubation of bacteria on plates in 6-well microtiter dishes

↓

Rinsing of unbound bacteria from plates with PBS

↓

Staining of bacteria with 0.1 % (w/v) crystal violet

↓

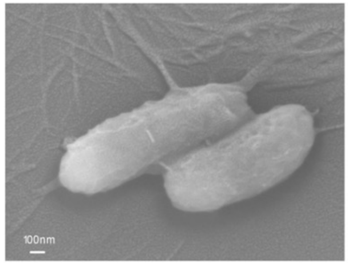
Rinsing redundand crystal violet

↓

Remobilizing crystal violet in 96 % ethanol

↓

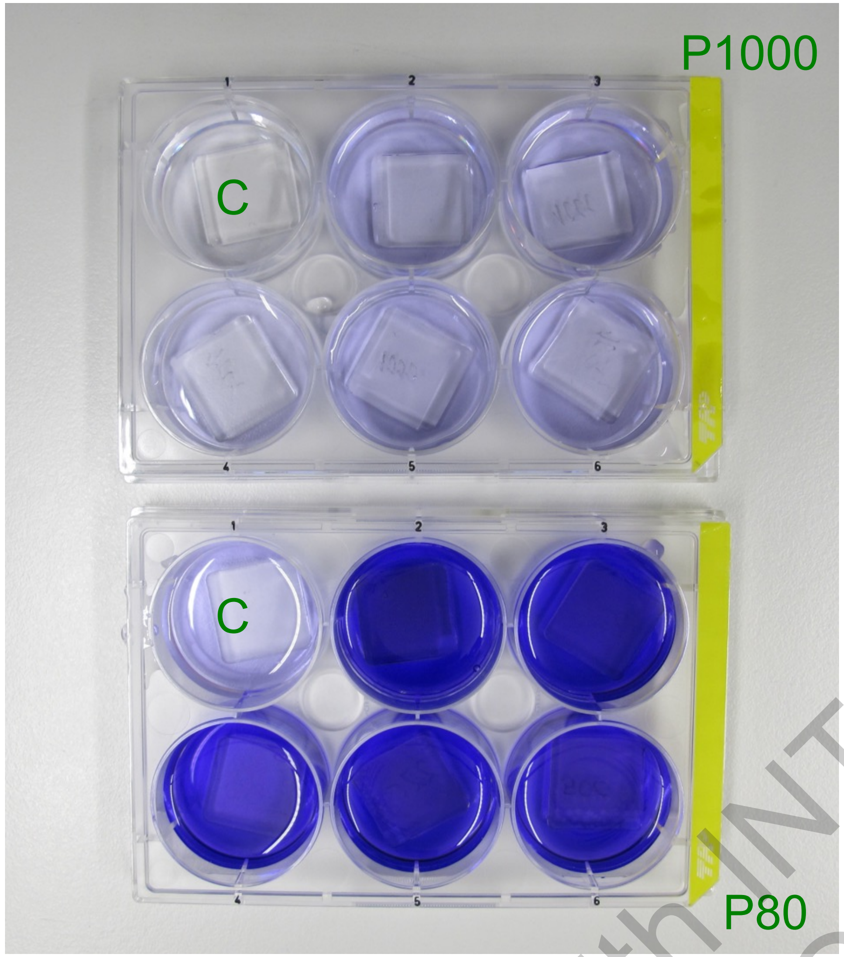
Measure absorbance of the solution at 620 nm (OD_{620})



Scanning electron microscopy

- produces images of a sample by scanning the surface with a focused beam of electrons
- the electrons interact with atoms, number of detected secondary electrons depends on specimen topography
- electron beam is scanned in a raster scan pattern
- resolution 1nm

Monitoring the adhesion



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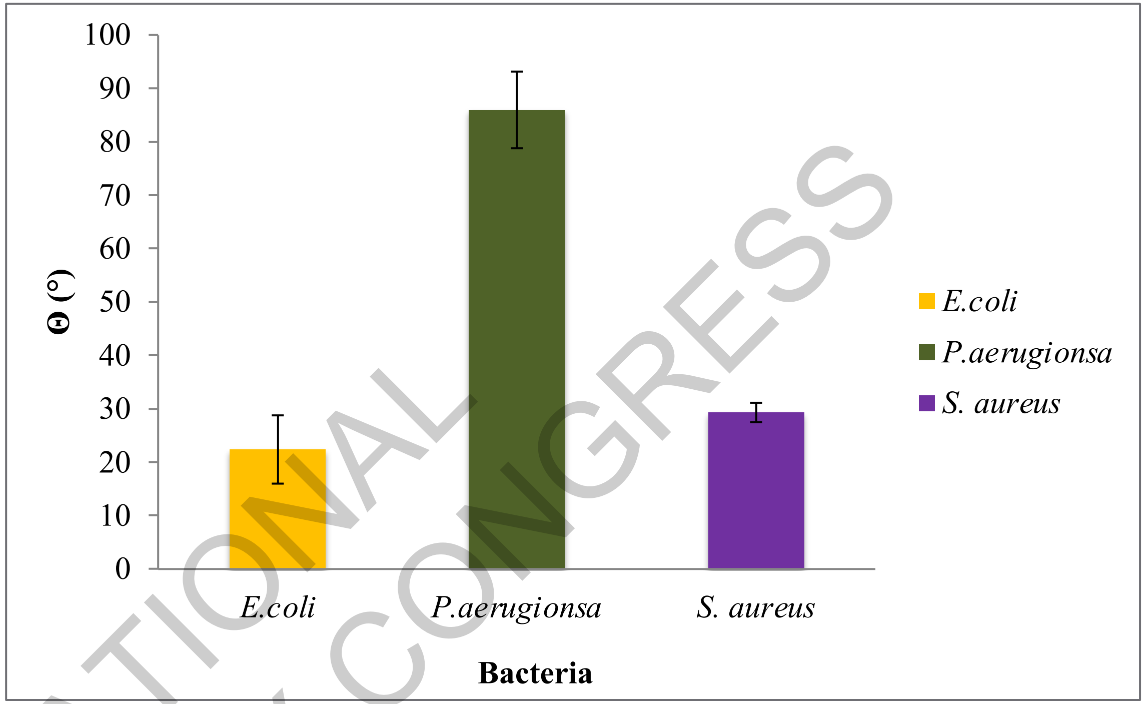
Measure absorbance of the solution at 620 nm (OD_{620})

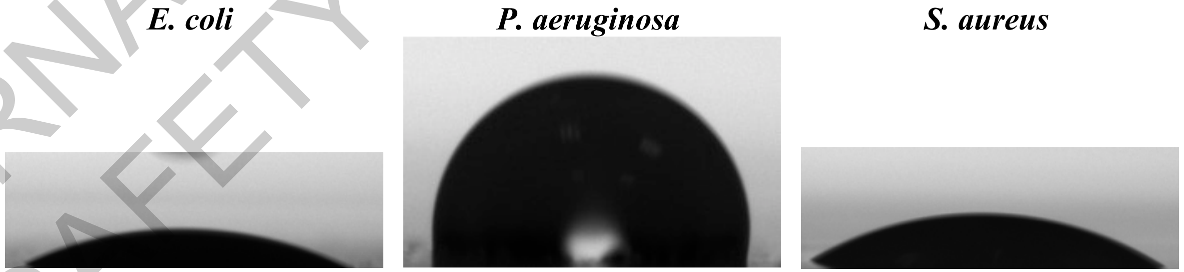
RESULTS

Characterization of microorganism:

- hydrophobicity
- surface charge density



Contact of liquid droplet with bacterial layer of *E. coli, P. aeruginosa* in *S. aureus*

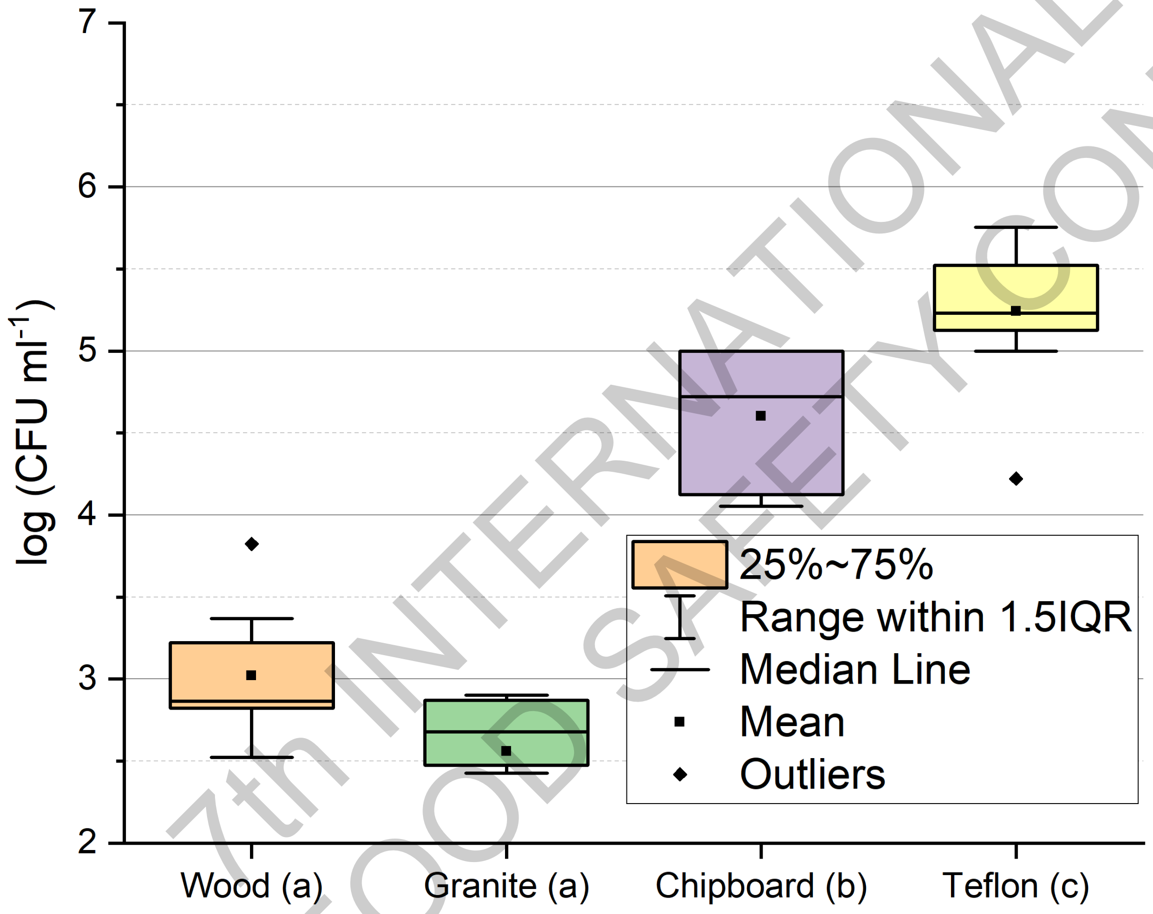


Bakteria	Zeta potencial (mV)	Volumen 1 % TiO_2 (mL)	Charge of bacterial cell (µekv/10^9 CFU)
P. aeruginosa	–50	25	0,312
E. coli	–151	6,3	0,078
S. aureus	–66	1,0	0,012

Results: Teflon, granite, beech wood, MFC

	Teflon	granite	beech wood	MFC
R_a/µm	0.36 ± 0.03	0.04 ± 0.004	2.34 ± 0.32	0.21 ± 0.01
R_q/µm	0.44 ± 0.04	0.08 ± 0.0005	2.90 ± 0.37	0.28 ± 0.02
Contact angle (°)	97.96 ± 3.41	53.57 ± 2.01	77.71 ± 8.0	65.1 ± 3.71
Zeta potential (mV)	−68.90 ± 1.47	-49.75 ± 0.96		-53.60 ± 3.55

Bacterial adhesion extent

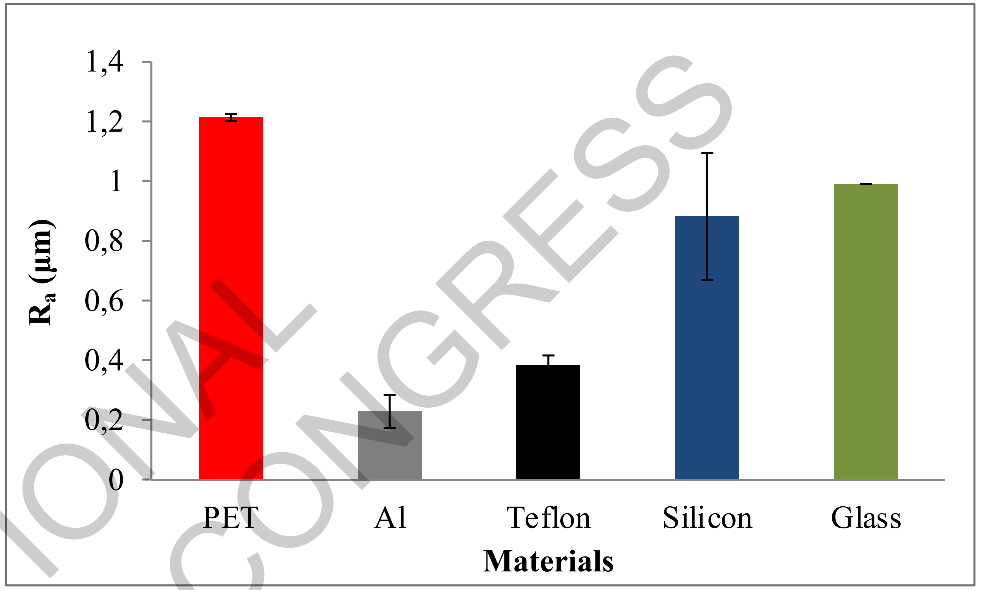


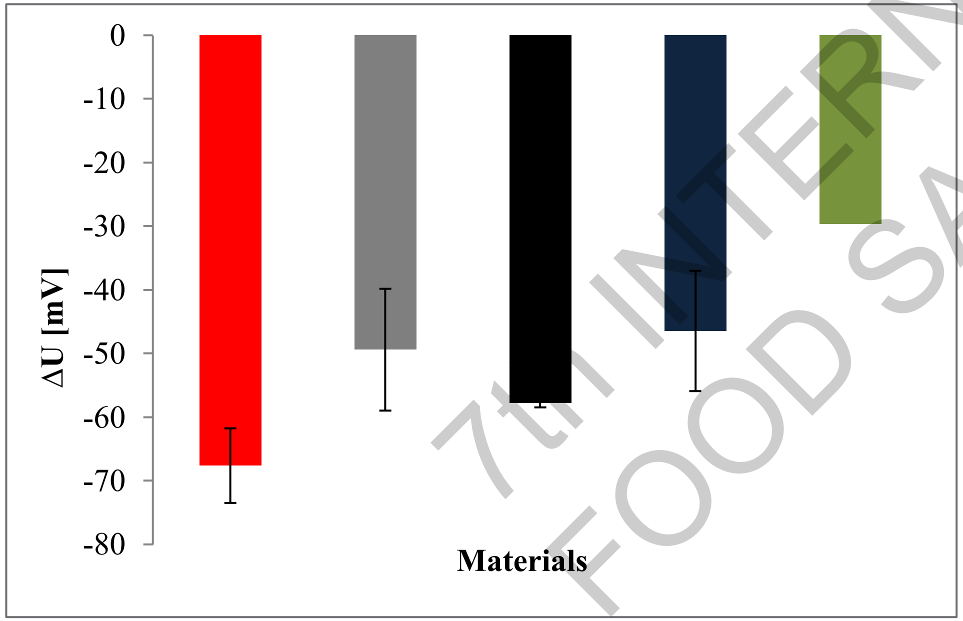
The lowest adhesion extent on granite surfaces

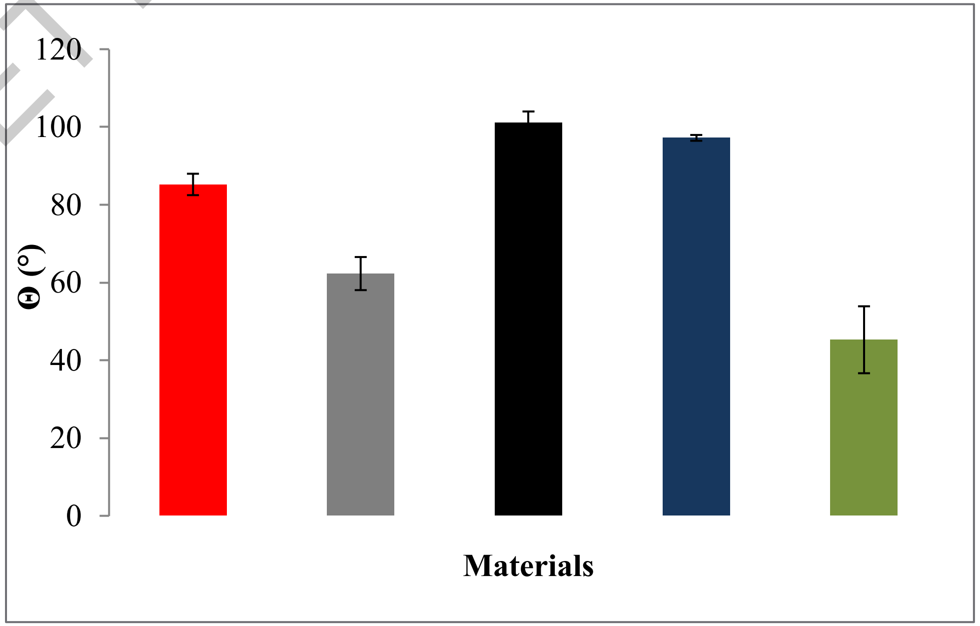
RESULTS

Characterization of material surfaces:

- Roughness R_a [μm]
- Hydrophobicity Θ [°]
- Zeta potencial ΔU [mV]



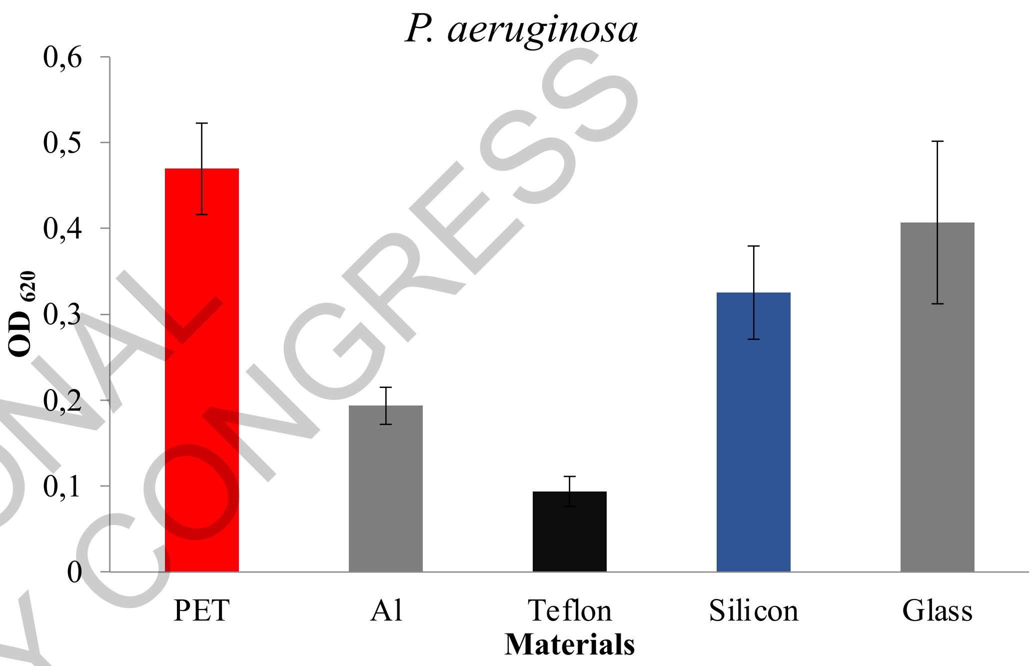


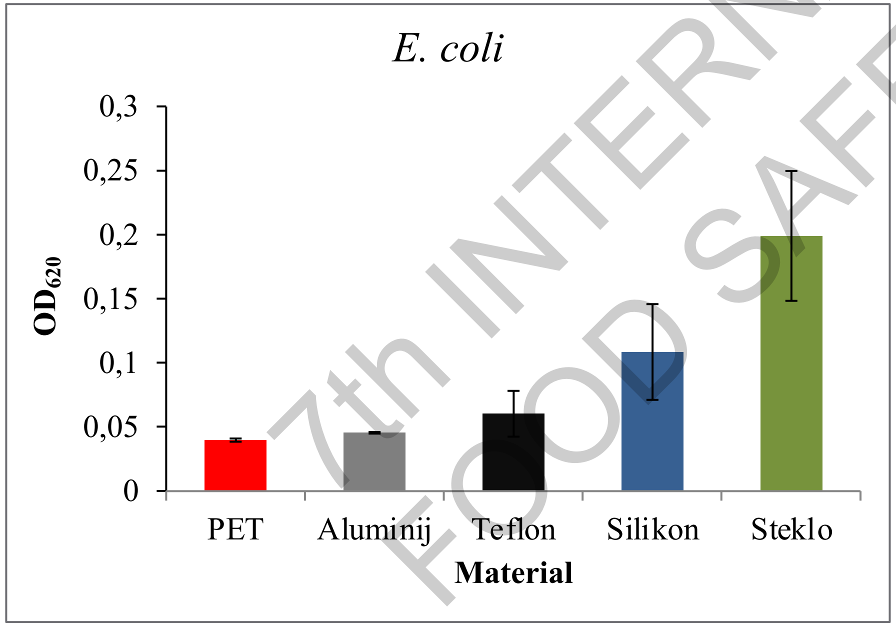


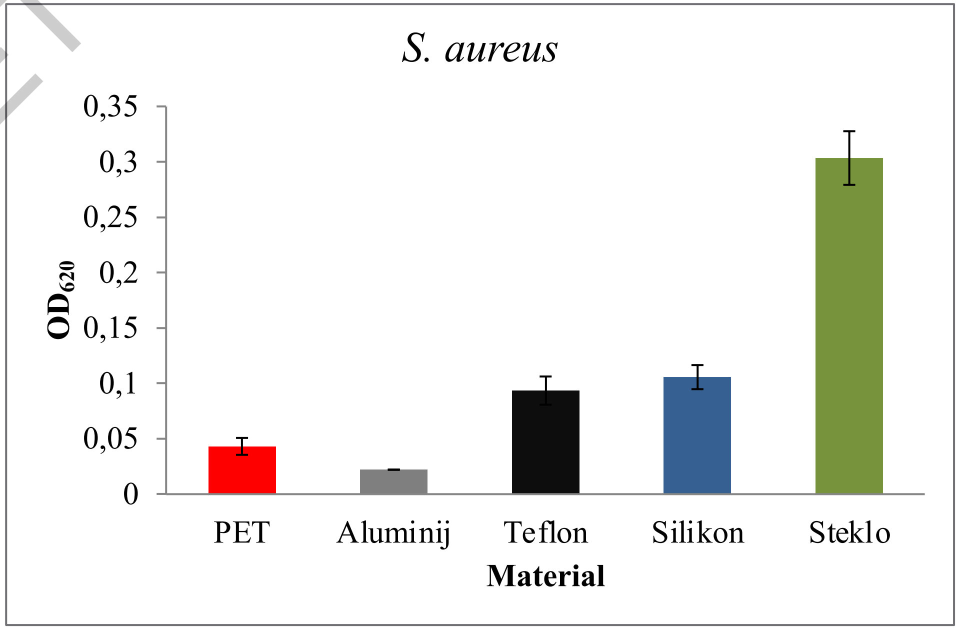
RESULTS

Measurement of OD_{620}

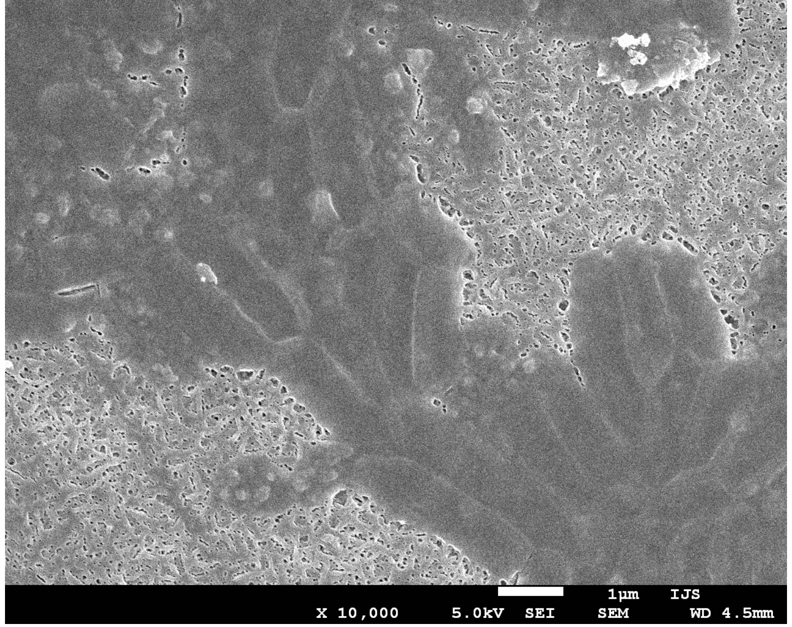
- Staining of adhered bacteria with crystal violet
- Measuring the absorbance of released crystal violet using spectrophotometer



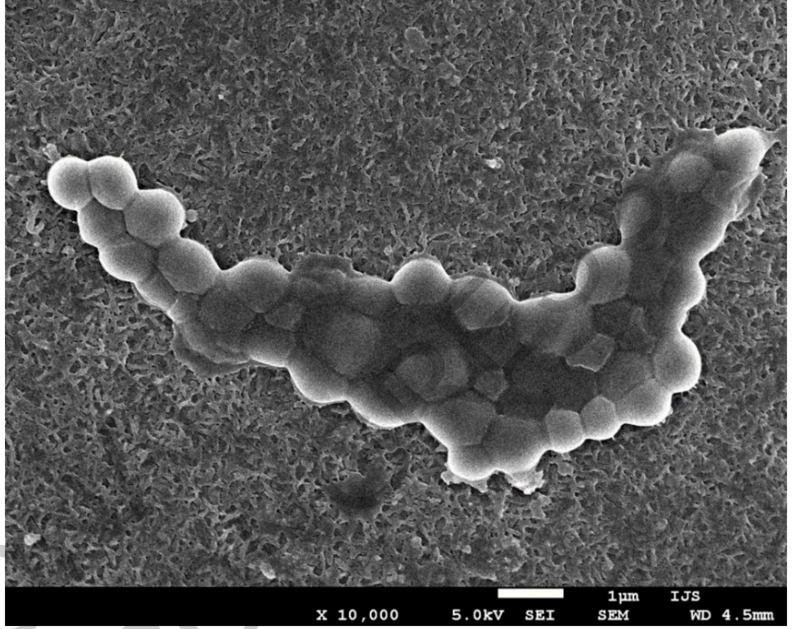




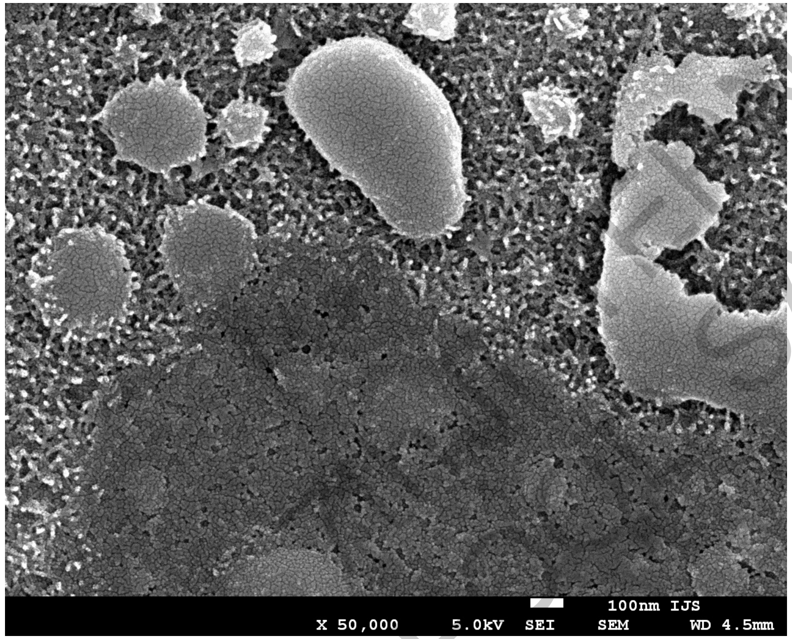
SEM IMAGES



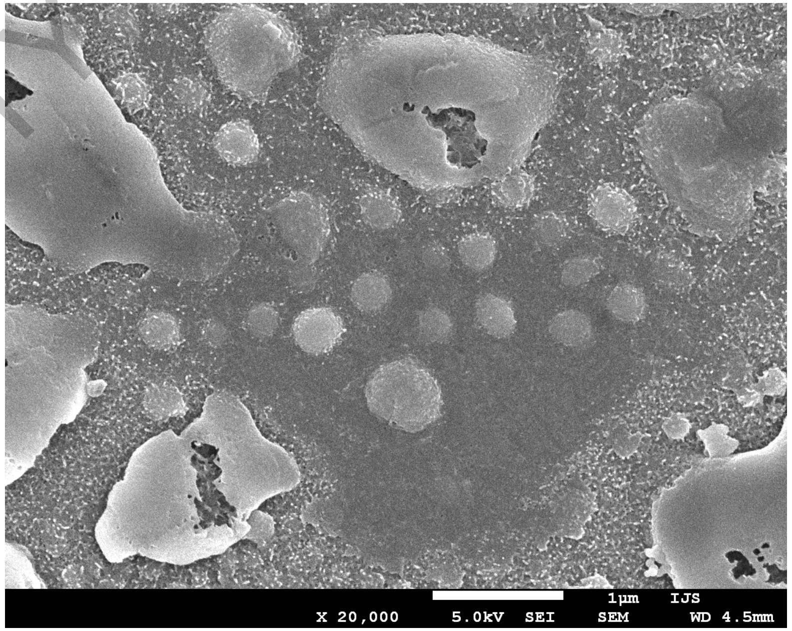
P. aeruginosa on Teflon



S. aureus on Teflon

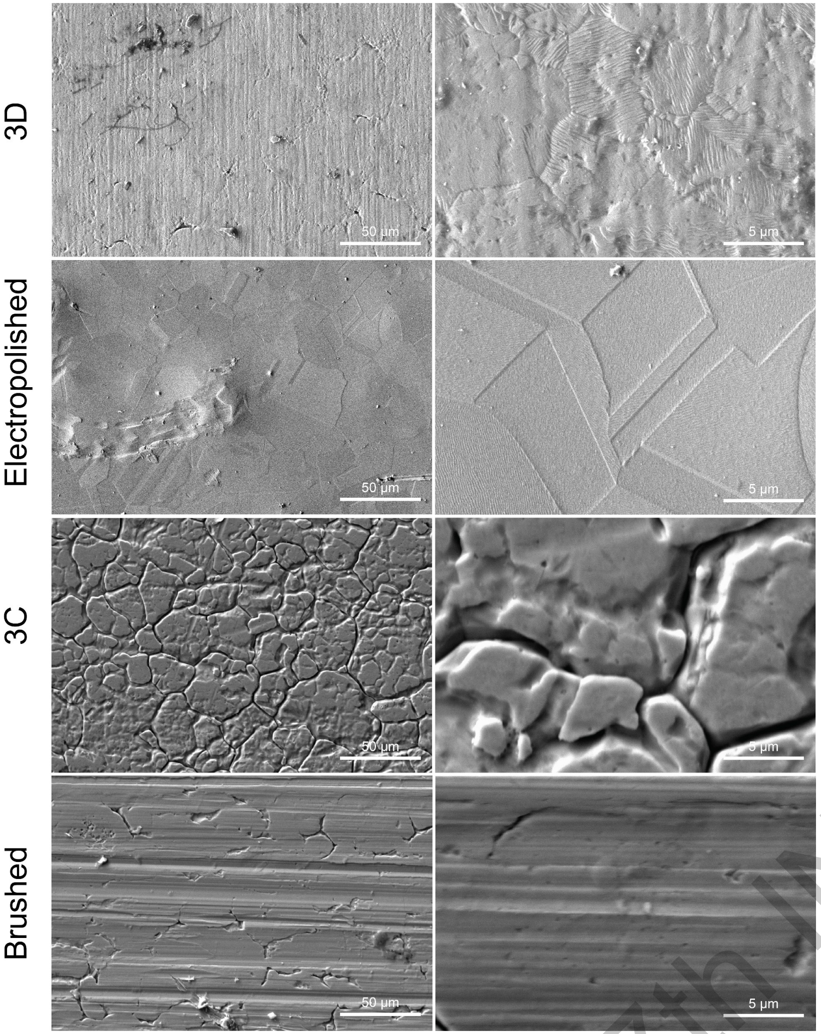


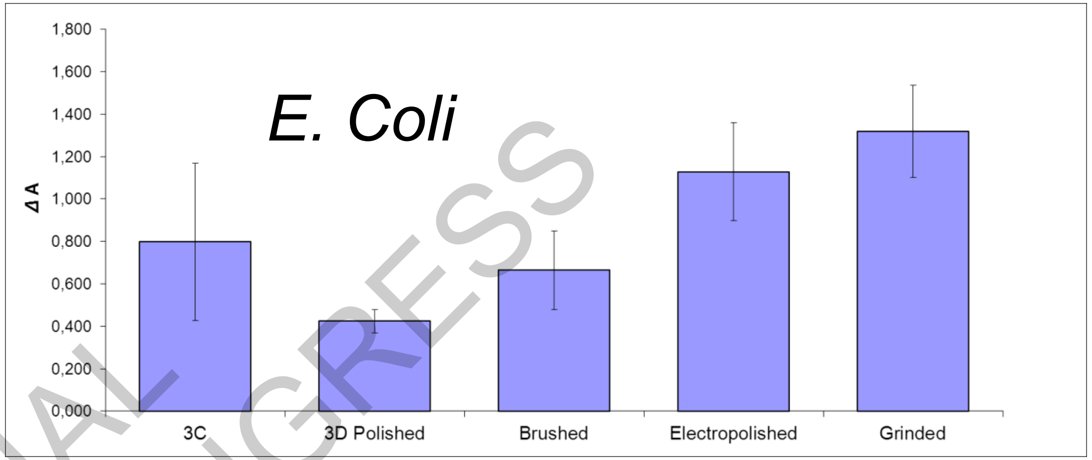
P. Aeruginosa on aluminium, 50.000x

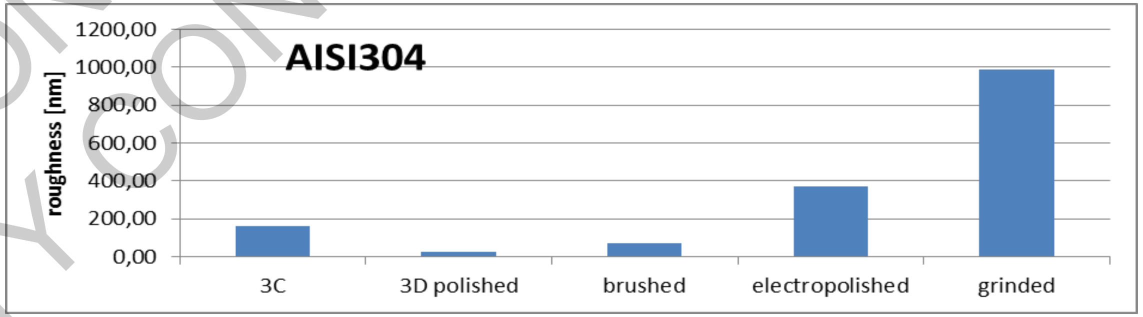


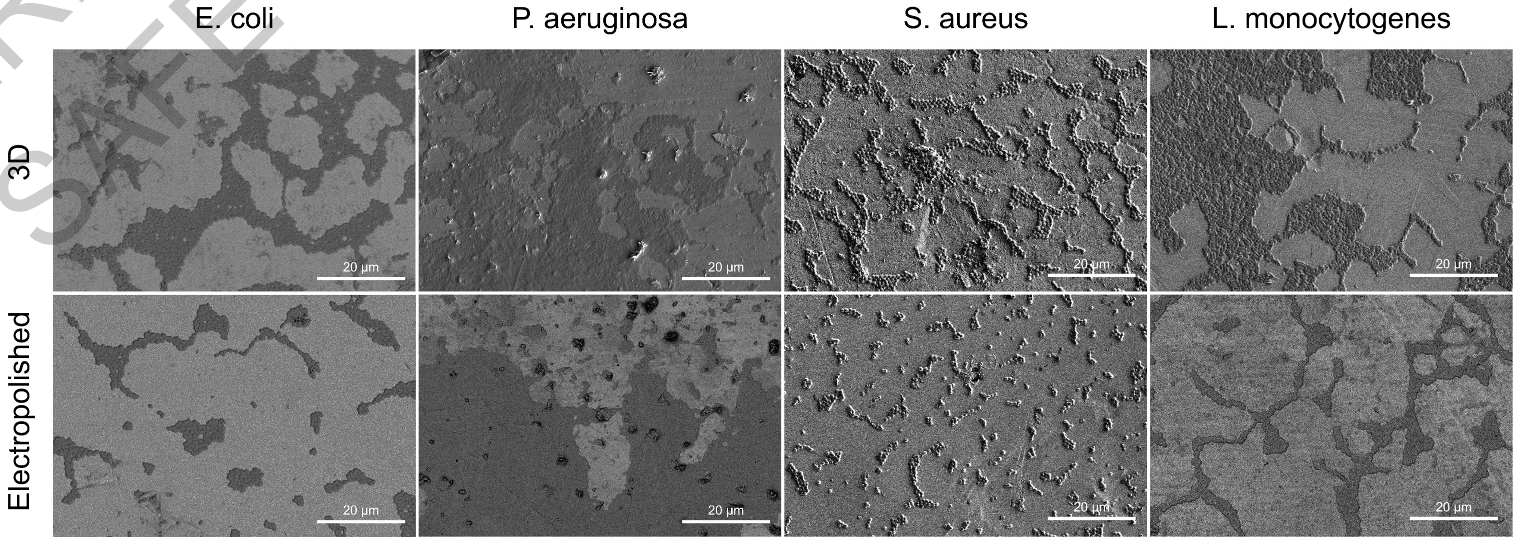
S. aureus on aluminium, 20.000x

Stainless steel surfaces



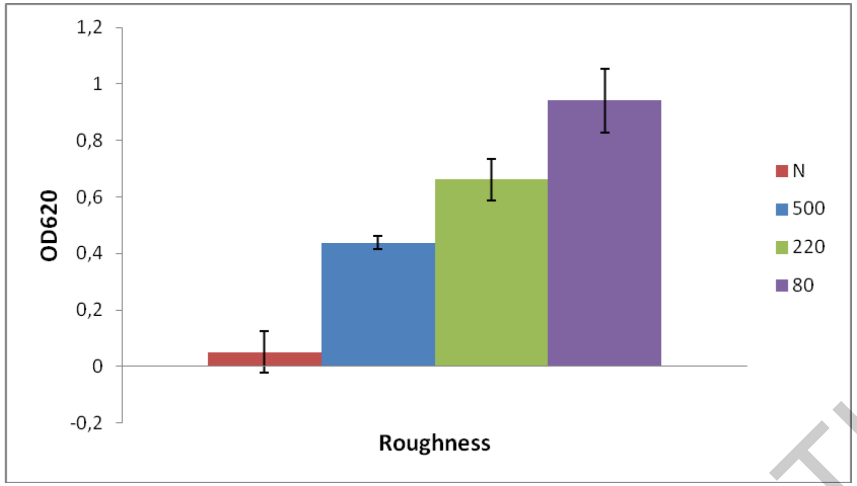






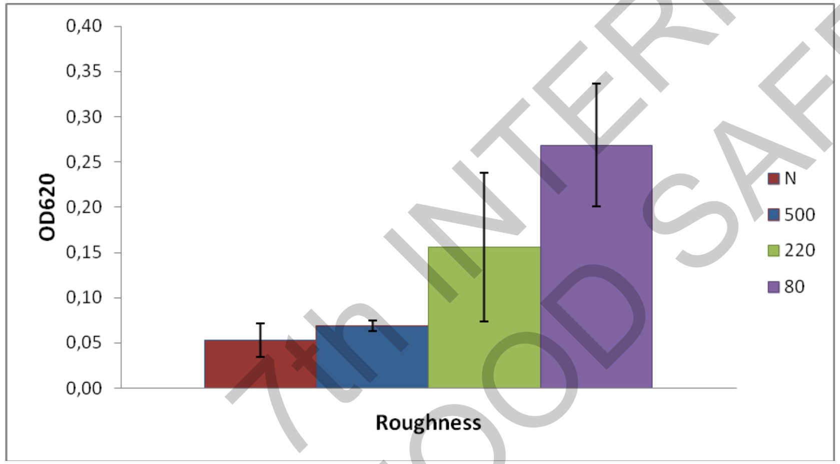
K. Bezek, D. Nipič, K. Godič Torkar, G. Dražić, A. Abram, J. Žibert, P. Raspor, K. Bohinc, Biofouling of stainless steel surfaces by four common pathogens : the effects of glucose concentration, temperature and surface roughness. *Biofouling*, 2019, vol. 35, iss. 3, 273-282

Glass surfaces

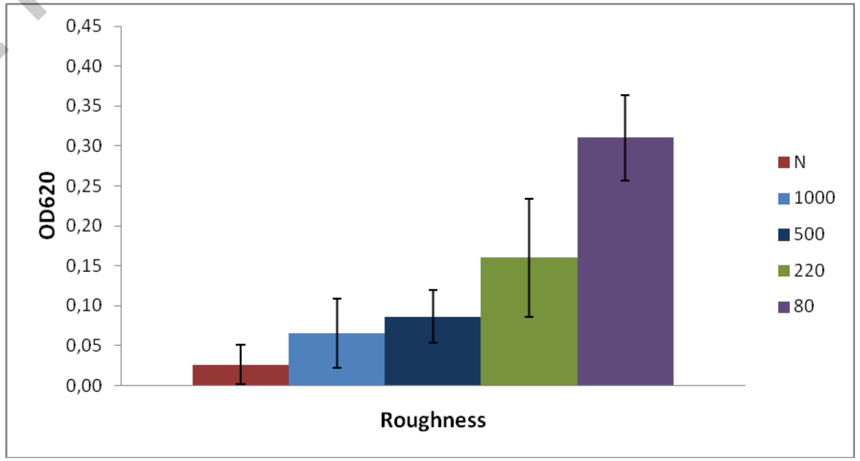


Pseudomonas aeruginosa
ATCC 27853

- measured absorbance of adhered cells on glass surfaces
- adhesion of bacteria increases with increasing roughness
- adhesion is bacteria specific



Staphylococcus aureus
ATCC 25923



Escherichia coli
ATCC 35218

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Conclusions

- Bacterial adhesion on kitchen surfaces
- The rate of adhered bacteria increases with increasing surface roughness
- The interplay between the increasing effective surface and increasing number of defects on the surface
- The adhesion rate depends also on the surface charge and hydrophobicity
- The lowest adhesion extent we observed on granite surfaces

Outlook: bacterial adhesion measurements on most kitchen top surfaces; comparison between different materials