Bacterial adhesion and biofilm formation on surfaces of variable roughness and hydrophobicity

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Introduction

Biofilm formation on surfaces in food production can deteriorate the quality of food products and be a hazard to consumers. An attractive approach in the fight against biofilm formation is the modification of surfaces to impede the first step in biofilm formation, namely bacterial adhesion. Sol-gel technology and the recent availability of organically modified silicas have lead to development of hybrid organic/inorganic glass ceramic coatings with specialised surface properties. Surface properties such as hydrophobicity, roughness and predisposition for fouling by protein are recognised as important in bacterial adhesion.

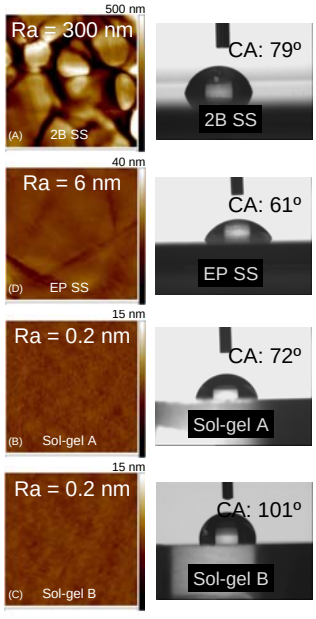
Objectives

In this study bacterial adhesion and subsequent biofilm formation on stainless steel (SS) and two nanostructured sol-gel surfaces with variable hydrophobicity was compared. The bacteria were enriched from minced pork and the microbial community were furthermore compared to bacteria from surfaces in the meat processing industry

Characterization of surfaces

Two sol-gel surfaces were compared to electro polished stainless steel (EP SS) and 2B finish stainless steel (2B SS). Hydrophobicity and roughness (Ra) of the surfaces were measured by contact angle measurements (CA) and atomic force microscopy (AFM). Fouling by bovine serum plasma was measured by quartz crystal microbalance (QCM) and mass estimated using Sauerbrey equation.

Surfaces	Mass adsorbed ($ng\ cm^{-2}$)
QCM SS	1009 ± 18
Sol-gel A	513 ± 265
Sol-gel B	508 ± 253



TappingMode AFM height images. Height scale 15-500 nm. A: scan size 10 x 10 µm². B-D: Scan size 2 x 2 µm²

→The roughness of Sol-gel surfaces and EP SS was 100 to 1000 fold lower than 2B SS

→ Sol-gel surfaces were more hydrophobic than stainless steel

→Less protein adsorbed to sol-gel surfaces compared to EP SS

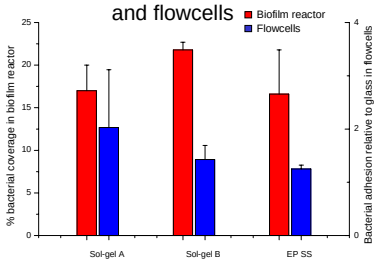
Bacterial adhesion

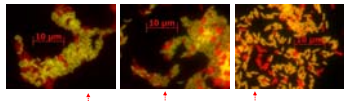
Significantly fewer bacteria adhered to sol-gel compared to 2B stainless steel. In the following, we compare sol-gel to polished steel to rule out the effect of surface roughness.
Adhesion of bacteria to EP SS, sol-gel A,and sol-gel B were quantified by microscopy of surfaces that had been incubated in a flowcell or in a biofilm reactor.

→Less bacteria adhere to sol-gel compared to the rougher 2B SS

→ No significant difference in adhesion on sol-gel surfaces and EP SS

Adhesion of bacteria in biofilm reactor and flowcells





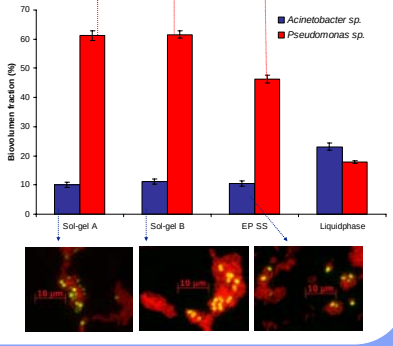
Community composition

The abundance of key organisms was determined in suspended biomass and biofilm from the biofilm reactor by fluorescence *in situ* hybridization (FISH) and image analysis.

→*Pseudomonas sp*. attached well to surfaces, while *Acinetobacter sp*. mostly attached to surfaces already colonized by other bacteria

→*Pseudomonas sp.* contributed less to the microbial community adhering to EP SS compared to sol-gel

Volume fraction of *Pseudomonas sp.* and *Acinetobacter sp.*



Microbial identification

	Biofilm reactor	Butcher table before cleaning	Butcher table after cleaning
Acinetobacter	7	3	1
Aeromonas	13		
Brochothrix		5	
Dermatococcus			1
Kocuria		3	27
Lactobacillus		2	
Lactococcus		4	1
Photobacterium		1	
Pseudomonas	11	10	1
Psychrobacter		4	1
Shewanella	1		

List of closest relatives to sequences obtained from a steel surface in a biofilm reactor and a steel table in a local butchers shop. Numbers indicate the number of clones within each group.

Bacteria from EP SS surfaces in the biofilm reactor were identified by 16S rRNA sequencing and compared to biomass from tables in a local butcher shop obtained before and after cleaning of the facilities.

→ Diversity in the bioreactor was low, but it contained 2 of the important bacterial groups found in the in the local butchers shop.

Conclusion

→Sol-gel coated surfaces reduce bacterial adhesion compared to 2B SS, which is commonly used in the food industry

→Biofilm formation is affected more by surface roughness than hydrophobicity (within the range studied here)

→The composition of bacteria on surfaces in the biofilm reactor was different from what was found in the liquid, indicating a selection of biofilm-forming bacteria on the surfaces.

→The bacterial community in the biofilm reactor is to some extent representative for what surfaces in a butchers shop are exposed to.