Microfluidic assay to investigate bacterial adhesion

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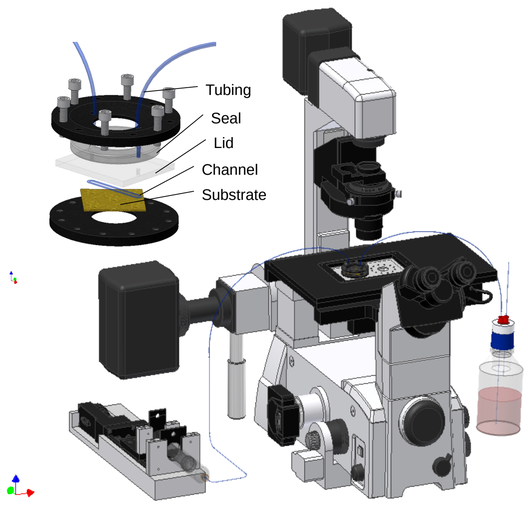
INTRODUCTION

Our approach

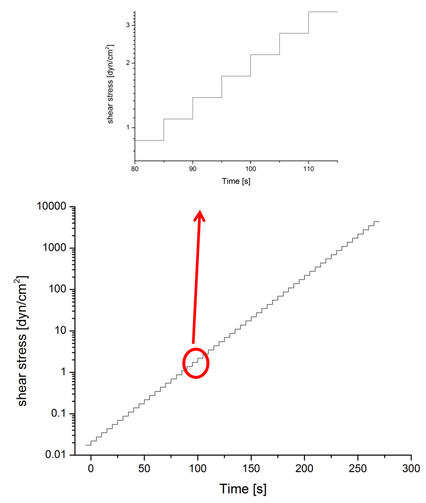
- Reveal the effect of surface properties on the adhesion strength of marine bacteria.
- Test organism: Marine bacterium *Cobetia Marina (*aerobic, gram-negative) used as a model system for marine biofilm formation
- Biofilms are important as they influence secondary colonization by invertebrates and algae[1].
- To optimize foul-release of bacteria we quantify the adhesion strength in a microfluidic assay[2].

Cobetia Marina imaged by SEM.

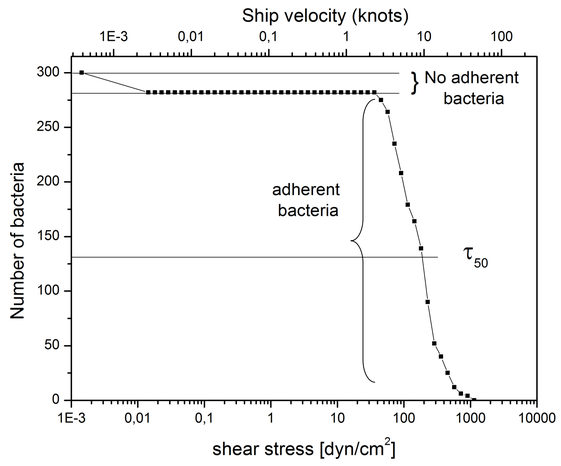


The setup: incubation microscope with syringe pump, microchannel and liquid reservoir .

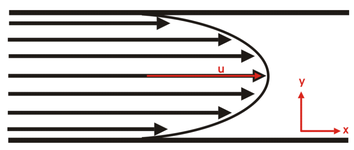
Application of a continuously increasing shear stress



Number of bacteria vs. shear stress



- The microchannel consists of PDMS (polydimethylsiloxane) and is mounted between a glass slide and the surface of interest. After the incubation time, the flow rate is stepwise increased via a syringe pump. Bacteria detachment is followed by video microscopy.
- The fraction which remains on the surface after application of small shear stresses up to 0.01 dyn/cm² gives the adherent cell fraction.
- The adhesion strength is determined as the shear stress needed to detach 50% of the adherent bacteria (τ_{50}).
- For the calculation of the shear stress, the flow volume and channel dimensions are taken into account [3-4].



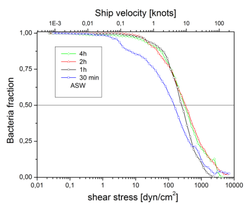
t = shear stress; μ = viscosity; Q = volumetric flow; h = channel height; w = channel width

$$\tau = \mu \frac{du}{dy} \quad (1)$$

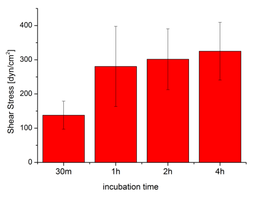
$$\tau = \frac{6 \cdot \mu \cdot Q}{h^2 w} \quad (2)$$

OPTIMIZATION OF PARAMETERS

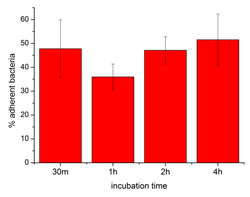
Optimization of incubation time



Bacterial removal curve



Average shear stress (τ_{50})

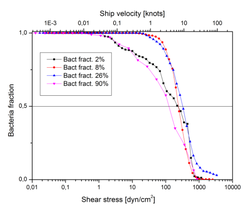


% adherent bacteria

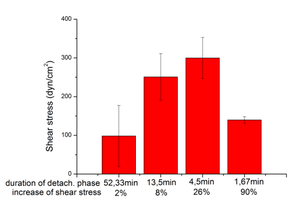
- Incubation time and shear rate were optimized in order to find the optimal assay parameters.
- Bacteria were left settle on Next glass for different times ranging from 30 min to 4 h.
- The percent of adherent bacteria barely depends on incubation time.
- Adhesion strength in contrast increases with the time.
- The settlement time does not strongly influence the shear stress if it is larger than 1hour.
- Conclusion: 2 h settlement time is the parameter that will be used for testing surfaces.

Optimization of shear rate

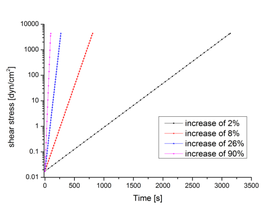
- Microfluidic experiments were carried out with an incubation time of 2 h.
- Different increases of shear stress were tested: 2, 8, 26 and 90%.
- Graphics show that the maximal value for the critical shear stress for removal is reached at 26% increase.
- This flow is chosen as best compromise between adaption and reliable removal at each data point.



Average bacteria fraction vs. shear stress



Average shear stress (τ_{50})

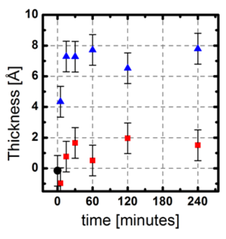


Different shear stress vs. time

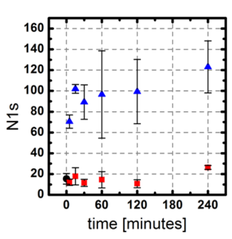
Conditioning of the surfaces by ASW and marine broth medium

- Assay time of 2 hours is found to be advantageous
- Question arises, can we afford to offer nutients in this time despite potential surface conditioning?
- Conditioning was measured on glass slides which were pre-incubated in both, ASW and MB media for different times ranging from 5 minutes to 4 hours.
- The graphic shows how the wettability of the surface is influenced by conditioning. After an immersion into ASW, glass slides become more hydrophilic. An immersion into MB causes a drastic change of the CA and the surfaces become more hydrophobic. Those effects occur within the first 30 minutes, afterwards the contact angles barely vary.
- Protein layer thickness and nitrogen composition of the pre-incubated samples were quantified by XPS. The thickness of the conditioning layer deposited from MB was ~7 Å and significantly thicker than the for the one for the sample incubated in ASW (~1 Å). In agreement to the contact angle values, after 30 minutes the thickness remained nearly constant. N1s XPS signals indicate a higher presence of nitrogen on samples incubated in MB, while incubated in ASW lack the presence of nitrogen.
- ASW seems to be a good choice for medium as conditioning is minimized

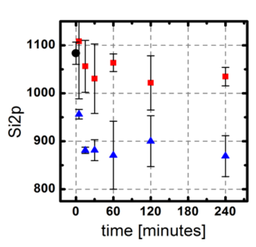
→ Conditioning in MB is likely to influence assay, effect on adhesion strength is currently under investigation



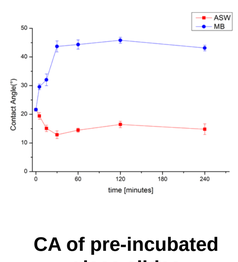
Layer thickness



Nitrogen composition



Silicon composition



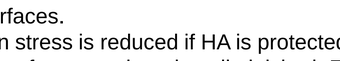
CA of pre-incubated glass slides

INFLUENCE OF SURFACE CHEMISTRY AND HYDRATION ON ADHESION

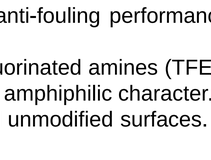
Influence of surface chemistry and wetting on adhesion

- Several different terminated SAMs were tested using the above described experimental parameters.
- Coatings have different wetting properties, but all surfaces have a similar SAM thickness except for PEG, which is slightly thicker.
- Chemical termination of the SAMs barely influenced both, adherent fraction and bacterial adhesion strength.
- The only exception is PEG2000-OH, which significantly reduced both, the fraction of adherent bacteria and the attachment strength.

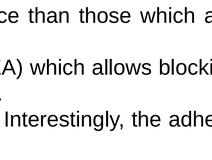
Surfaces	PEG	HUDT	AUDT	DDT	FUDT
Contact angle/°	30 ± 1	38 ± 1	54 ± 1	106 ± 1	113 ± 2
Thickness /Å	30 ± 2	10 ± 1	16 ± 1	13 ± 1	16 ± 1



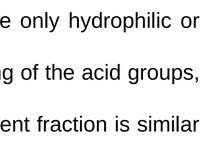
Waterdrop on each surface



Average shear stress (τ_{50})



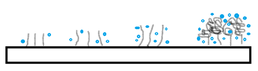
% adherent bacteria



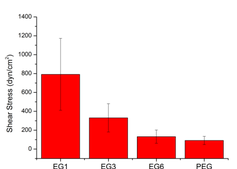
Average bacteria fraction vs. shear stress

Influence of hydration on adhesion

- Influence of hydration on adhesion of Cobetia has been studied.
- Trend towards weaker adhesion with increasing number of ethylene glycol units.
- Bacteria were adhered equally on the surfaces except for PEG-OH where approximately only half as many bacteria adhered compared to the other linear homologues.
- On EG_1OH bacteria are roughly at least 2x stronger attached than for the longer homologues.
- Adhesion strength is reduced with an increasing ability of the surfaces to bind water.
- Results are in agreement with Ulva spore adhesion [5].



Surfaces	EG1	EG3	EG6	PEG
Contact angle/°	28 ± 1	30 ± 1	32 ± 1	30 ± 1
Thickness /Å	15 ± 1	22± 1	26 ± 1	30 ± 2



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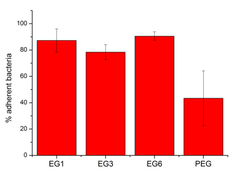
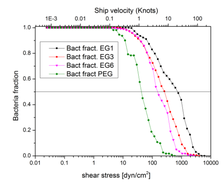


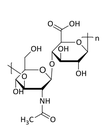
% adherent bacteria



Average bacteria fraction vs. shear stress

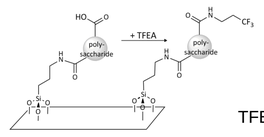
Adhesion to amphiphilic polysaccharides

- Polysaccharides are potential candidates for non-fouling coatings, since they are highly hydrophilic due to their free hydroxyl-groups [6].
- HA and CS were chosen, since both have only one carboxylic-acid moiety per disaccharide unit. The use of CS is bioinspired due to its presence in fish mucus and its potential contribution to protection of the skin of fish [7].
- Polysaccharides have only weak antifouling potential in the marine environment as they bind bivalent ions and thus loose their hydration.
- amphiphilic surface-coatings have a higher anti-fouling performance than those which are only hydrophilic or hydrophobic [8].
- HA and CS were capped with hydrophobic fluorinated amines (TFEA) which allows blocking of the acid groups, shifting of the contact angle and achieving an amphiphilic character.
- Clear difference between amid modified and unmodified surfaces. Interestingly, the adherent fraction is similar on all surfaces.
- Adhesion stress is reduced if HA is protected by hydrophobic groups.
- For CS performance is rather diminished. This could be due to the reason that the sulfate group itself causes the good foul removal properties for the biomimetic CS and thus the material is well chosen by nature.

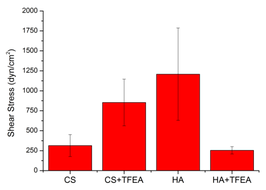


CS: Chondroitin sulfate

HA: Hyaluronic acid



TFEA: 2,2,2-trifluoroethylamine F_3C NH_2



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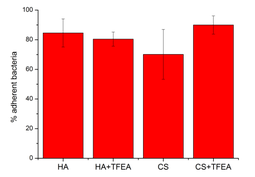
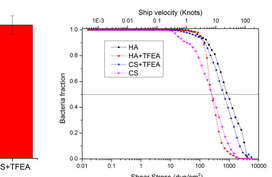


% adherent bacteria



Average bacteria fraction vs. shear stress

HA	HA+TFEA	CS	CS+TFEA
<10°	~30°	12°	~35°

CONCLUSIONS

- A microfluidic attachment strength assay for bacteria has been developed.
- Best parameters were found: 2h incubation time and 26% shear rate.
- Hydration of the surface is important and lowers bacterial adhesion strength.
- The termination of the SAM barely influenced bacterial adhesion strength and settlement except for PEG.
- Polysaccharide were investigated as potential antifouling coating candidates.

LITERATURE

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