A microfluidical assay to quantify the adhesion strength of marine microorganisms

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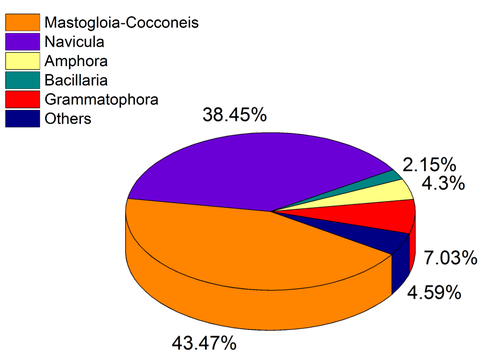
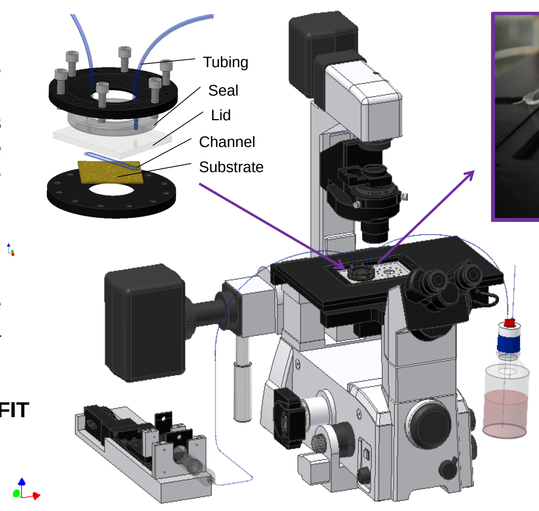
INTRODUCTION

Approach and motivation

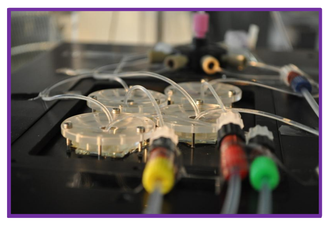
- Reveal the effect of surface properties on the adhesion strength of microorganisms.
- Diatoms are frequently observed biofoulers among the first colonizing species and are prevalent on modern foul release technologies.
- Test organism: Diatom *Navicula perminuta*, used as a model system for marine biofilm formation.
- To optimize foul-release of diatoms we quantify the adhesion strength in a microfluidic assay.[1]

Diatom population identified in field tests at FIT

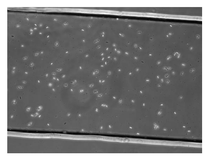




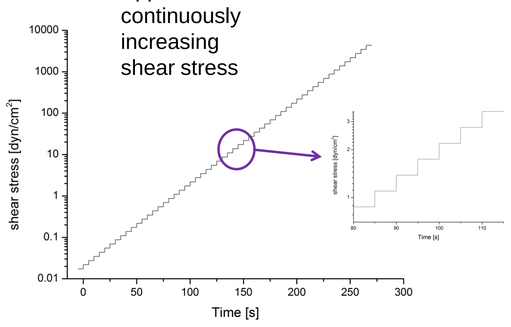
The setup: Incubation microscope with syringe pump, microchannels and liquid reservoir



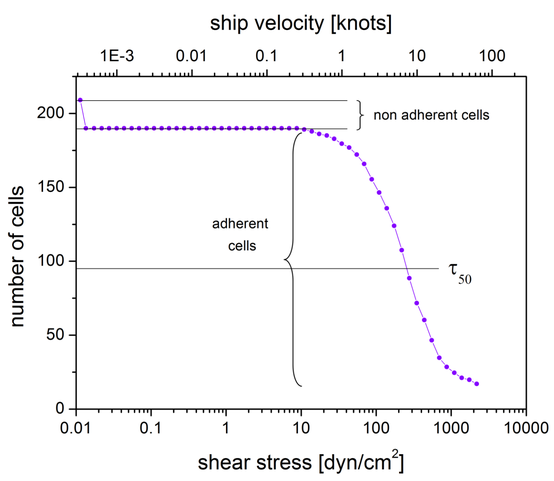
Navicula perminuta cells in a microfluidic channel



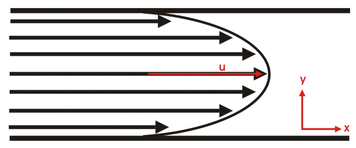
Application of a continuously increasing shear stress



Number of microorganisms vs. shear stress



- Microchannels consist of PDMS (polydimethylsiloxane) and are mounted between a glass slide and the surface of interest. After the incubation time the flow rate is stepwise increased via a syringe pump. Algae cell detachment is followed by video microscopy.
- Fraction which remains on the surface after application of small shear stresses up to 0.01 dyn/cm² gives the adherent cell fraction.
- **Determination of adhesion strength: Shear stress needed to detach 50% of the adherent microorganisms (τ_{50})**
- Calculation of shear stress: flow volume and channel dimensions are taken into account.[2-3]



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

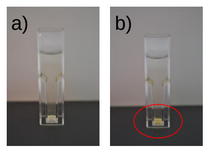
$$\tau = \frac{6 \cdot \mu \cdot Q}{h^2 w} \quad (2)$$

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

OPTIMIZATION OF ASSAY PARAMETERS

About *Navicula perminuta*

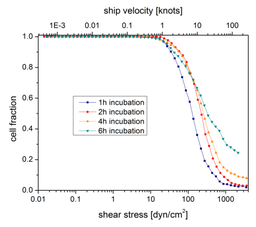
- Class of pennate diatoms, size of 12x5 µm, unicellular, also exists in colonies.
- Reaches surface by gravitation or convection, no active approach to a surface.
- Secretion of EPS through raphes, an elongate slit → cell-substratum adhesion, cell motility on substrates called gliding and colony formation.[4]



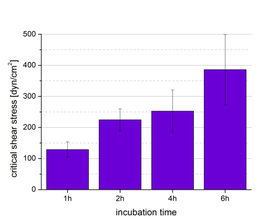
a) freshly vortexed
b) after few minutes

Optimization of incubation time

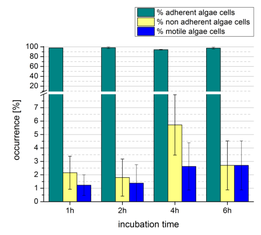
- Incubation time was optimized in order to find the optimal assay parameters.
- Algae cells were left to settle on Nexterion® glass for different times ranging from 1 to 6 h.



Algae cells removal curve



Average critical shear stress (τ_{50})

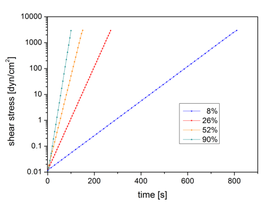


Distribution algae cells [%]
(motile cells = non adherent cells)

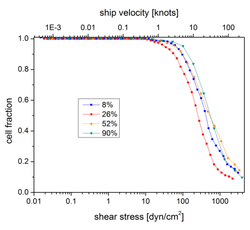
- → Percent of adherent algae cells barely depends on incubation time
- → Adhesion strength in contrast increases with time
- → Removal of algae cells after 6 h incubation is incomplete

Optimization of shear rate

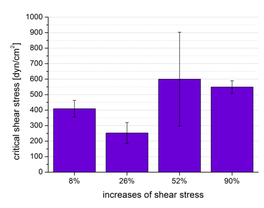
- Microfluidic experiments were carried out at an incubation time of 4 h as compromise of total assay duration and attachment strength.
- Different increases of shear stress were tested: 8, 26, 52 and 90% which results in total experiment durations of 15, 5, 1.5, 1 min → each step in the ramp: 5 s for 26, 52, 90% and 15 s for 8%.



Shear stress increase vs. time



Algae cells removal curve

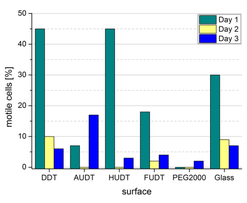


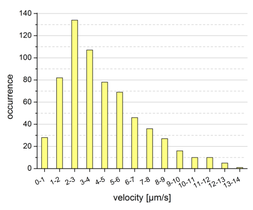
Average critical shear stress (τ_{50})

- → Adhesion strength increases with decreasing assay duration as the removal process takes time and the flow is increasing faster than the removal takes place
- → Increasing adhesion at 8% flow increase (total assay duration of 15 min) is due to adaption of the diatoms to the shear flow and thus enhanced adhesion
- → Flow increase of 26% was chosen as it yielded the most reliable data

Investigation of motility and velocity characteristics of *Navicula perminuta* on surfaces with different wettability

- *Navicula perminuta* cells are able to glide over a surface after reaching it.[4]
- Question arises: do surface properties affect how many algae cells move and how fast?
- Motility was investigated on chemically different SAMs and Nexterion® glass on three different days, under static conditions and with an increasing flow.
- Velocity evaluation shows an average of all surfaces (FUDT, DDT, AUDT, HUDT, PEG2000 and Glass; see right table for surface properties).





- → Motility does not appear to be an useful indicator of surface preference, what has also been shown testing silicone surfaces[5]
- → During the detachment assay motile *Navicula* move at a constant speed up to 150 dyn/cm². After they stop gliding they can stay attached up to 2500 dyn/cm²

INFLUENCE OF SURFACE CHEMISTRY AND HYDRATION ON ADHESION

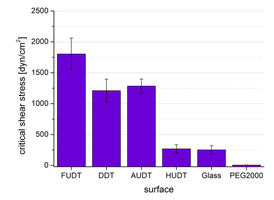
Single species tests

Influence of surface chemistry and wetting on adhesion

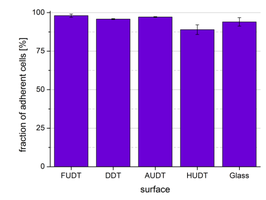
- SAMs with different chemical termination were compared using described experimental parameters.
- Coatings have different wetting properties with a similar SAM thickness, except PEG2000, which is slightly thicker.

Surfaces	FUDT	DDT	AUDT	HUDT	PEG2000
Contact angle/°	112 ± 2	104 ± 2	60 ± 2	33 ± 1	30 ± 1
Thickness/Å	16 ± 1	13 ± 1	16 ± 1	13 ± 1	32 ± 1
Waterdrop on each surface					

FUDT: HS-$C_{11}OC_7F_{12}CF_3$, DDT: HS-$C_{11}CH_3$, AUDT: HS-$C_{11}NH_2$, HUDT: HS-$C_{11}OH$, PEG: HS-$C_{11}(OC_2H_4)_{2000}OH$

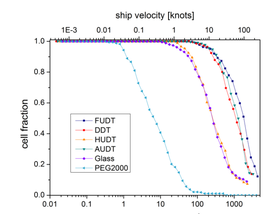


Average critical shear stress (τ_{50})



Adherent *Navicula* [%]

- → Chemical termination of a surface has a strong effect on attachment strength, but almost no effect on adherent fraction
- → Non adherent cells on PEG assemble to clumps, hence are not countable
- → Weak adhesion of algae cells on PEG and HUDT and strong adhesion on hydrophobic surfaces, in agreement with *Ulva linza* results[6]
- → Shear stress trend goes in agreement with studies of *Cobetia marina* bacteria [not yet published]



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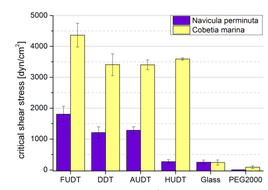


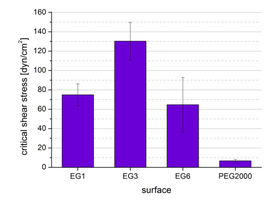
Navicula vs. *Cobetia*

Influence of hydration on adhesion

- Coatings with different hydration properties and their effect on adhesion strength have been studied.

Surfaces	EG1	EG3	EG6	PEG2000
Contact angle/°	28 ± 1	30 ± 1	32 ± 1	30 ± 1
Thickness/Å	15 ± 1	22 ± 1	26 ± 1	32 ± 1
Hydration				

EG1: HS-$C_{11}(OC_2H_4)_1OH$, EG6: HS-$C_{11}(OC_2H_4)_6OH$



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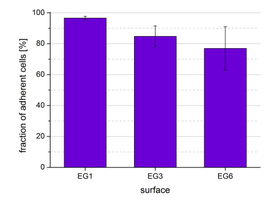
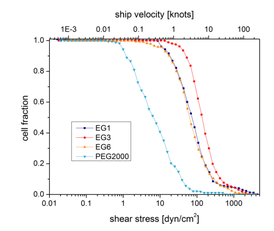
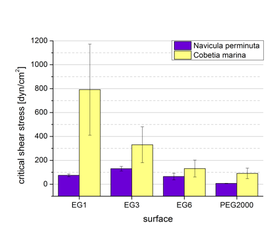


Adherent *Navicula* [%]

- → Number of ethylene glycol units has no strong influence on adhesion strength, except for PEG
- → Adhesion strength is equally on EG1 and EG6, adherent fraction in contrast decreases with increasing hydration degree of EG
- → EG3 shows twice as many algae cells adhered compared to the other linear homologues
- → PEG2000 shows a weak adhesion of algae cells
- → Difference in adhesion strength between EG1/EG6 is not as strong as for bacteria *Cobetia marina*



Algae cells removal curve

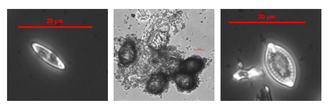


Navicula vs. *Cobetia*

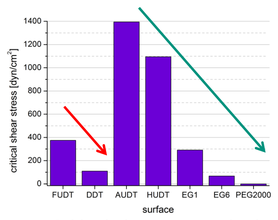
Comparison between laboratory and real marine world

- Testing of surfaces with different wetting and hydration properties (in collaboration with Prof. G. W. Swain, FIT, Melbourne, test side: EELS)

In contrary to lab: mixed population e.g.:



Navicula *Peritrich* *Mastogloia*



Critical shear stress (τ_{50})

- → EG1/EG6: in agreement with lab trend for *Cobetia* and *Ulva* spore adhesion[7]: adhesion decreases with increasing hydration degree of EG
- → Disagreement to lab: trend of very hydrophobic SAMs

CONCLUSIONS

- A microfluidic attachment strength assay for marine microorganisms has been developed.
- Best parameters were found: 4 h incubation time and 26% increasing shear rate.
- Motility and velocity of algae cells do not depend on chemical termination of a surface.
- Chemistry of the surface is important and can lower algae cell adhesion strength.
- Hydration degree of the SAM has no strong influence on algae cell adhesion strength except for PEG2000, on the contrary adherent algae fraction decreases with increasing hydration degree.
- Results of field work show agreement in the hydration trend but differences in the wettability trend

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