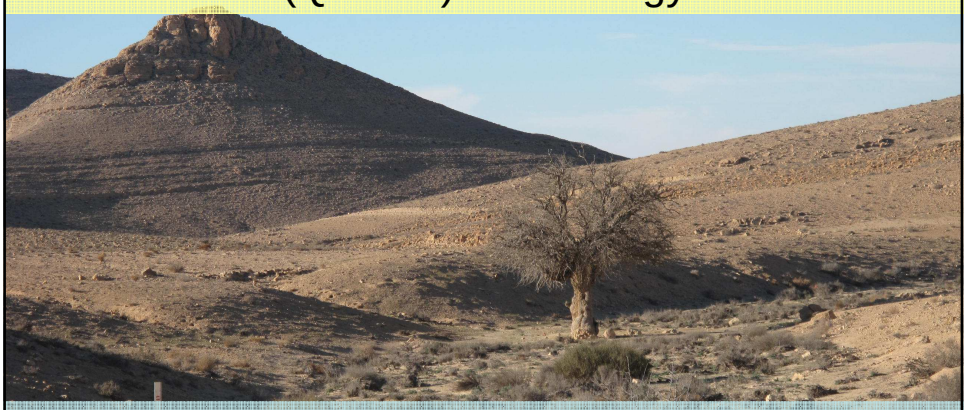
Biofouling Studies Using Quartz Crystal Microbalance with Dissipation Monitoring (QCM-D) Technology



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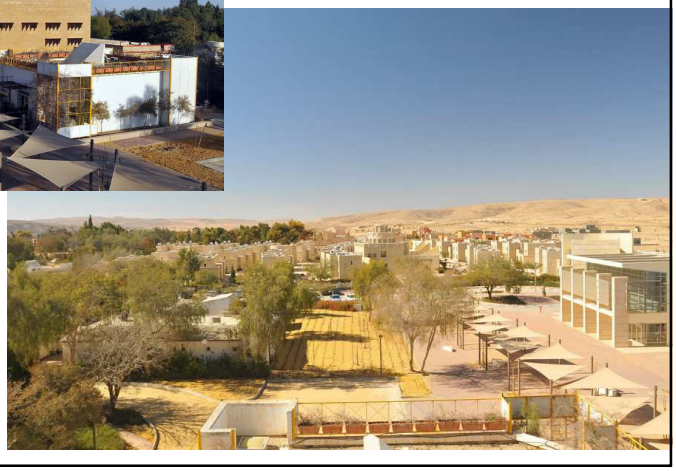

- **Department of Environmental Hydrology and Microbiology**
- **Department of Desalination and Water Treatment**

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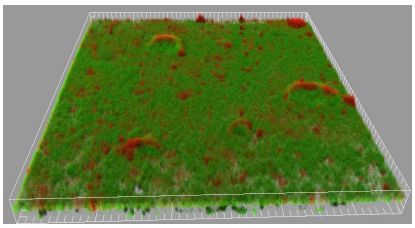
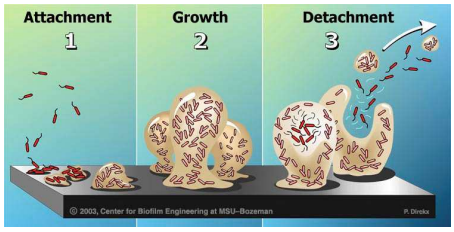
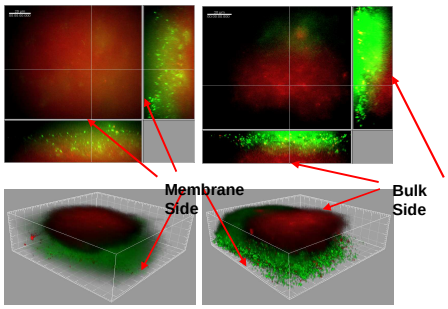
- **Technology of Desalination and Water Treatment**
- **Membrane Science and Technology**

Microbial biofilms





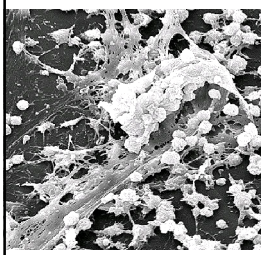
Bacteria form aggregates in a self produced EPS on membranes



EPS: The house of cells

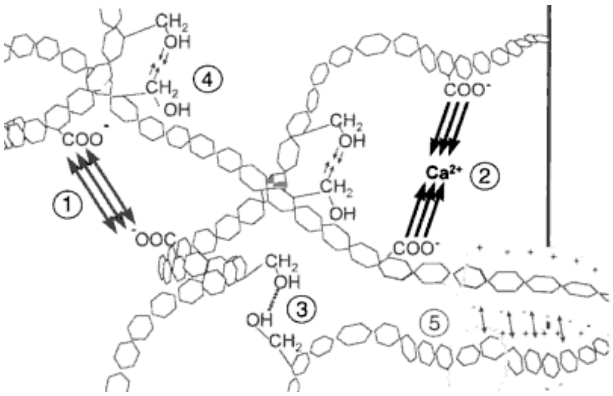
EPS are metabolic products, resulting from active bacterial secretion, shedding of cell surface material.

EPS determine the immediate environment and life conditions of biofilm cells:



- Filling and forming the space between the cells affecting porosity, density, water content, charge, hydrophobicity, sorption properties, and mechanical stability
- Influencing mass transport and serving as carbon and energy reserves during starvation
- Retarding penetration and sequester antimicrobial agents

What are the forces which keep the matrix together?



Interaction between EPS molecules.

1.repulsion of two carboxylic groups

2.attraction of two carboxylic groups by divalent cation

3.hydrogen bond

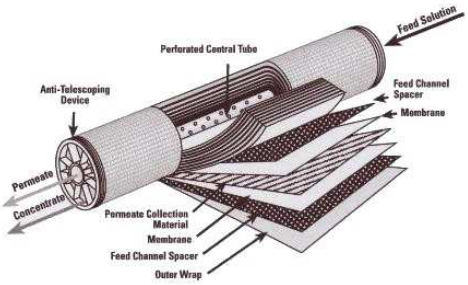
4.electrostatic attraction

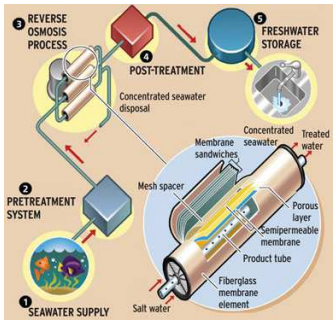
5.dispersion forces

Ashkelon Desalination Plant

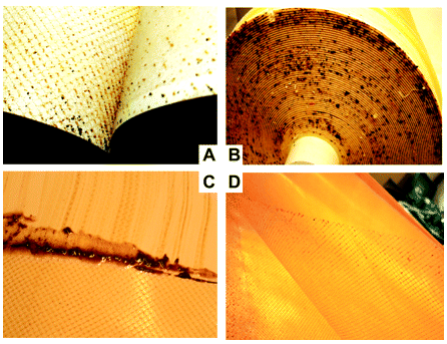
Ashkelon
108,000,000 m^3/year
Largest RO plant in the world

Our research on seawater desalination



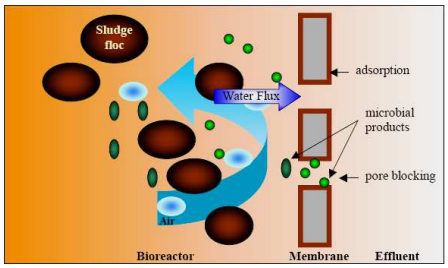


Type of foulant	Most susceptible stage of NF/RO
Scaling/silica	Last membrane in last stage
Metal oxides	First membranes of first stage
Colloids	First membranes of first stage
Organic	First membranes of first stage
Biofouling (rapid)	First membranes of first stage
Biofouling (slow)	Throughout the whole installation



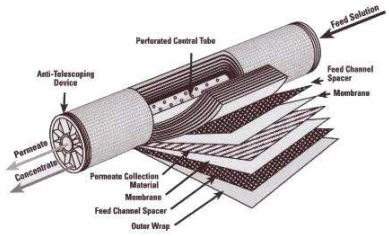
Fouling in membrane processes

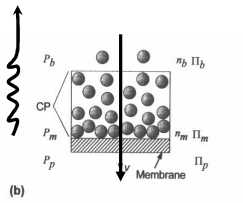
Microfiltration, ultrafiltration



- Adsorption of foulants to the membrane surface and pore blockage
- Increase in hydraulic resistance

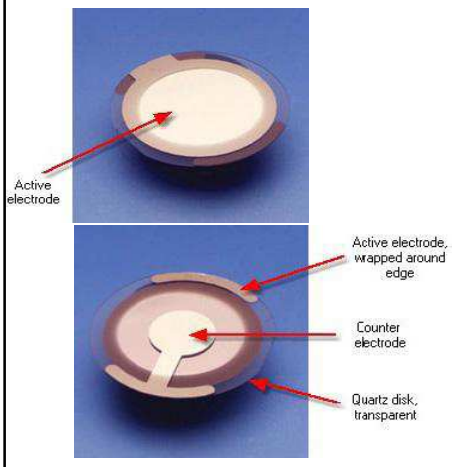
Reverse osmosis, nanofiltration

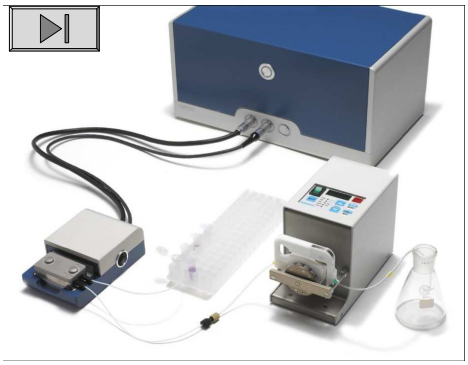




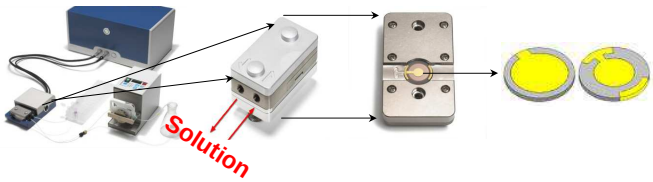
• Hoek and Elimelech ES&T 2003; Herzberg and Elimelech JMS, 2007*

Structural, viscoelastic properties and mass changes in real-time using quartz crystal microbalance with dissipation technology (QCM-D)

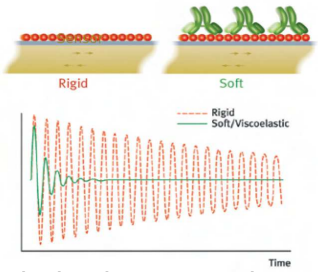




QCM-D



In the QCM-D, adsorbed mass (ng/cm^2 sensitivity) is measured as changes in oscillating frequency of the quartz crystal (F), and the energy dissipation (D), provides insights regarding viscoelastic and structural properties of adsorbed layers.



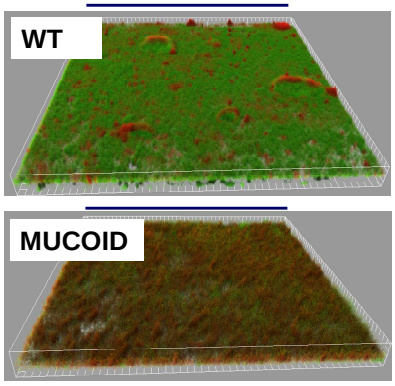
Dissipation energy loss

Case study I: The effect of alginate on EPS adherence and viscoelasticity

- EPS extraction and characterization from a *Pseudomonas aeruginosa*
  - Model strains for EPS "production": *Pseudomonas aeruginosa* PAO1 and its isogenic mucoid mutant PAO*mucA22*, PDO300[1]

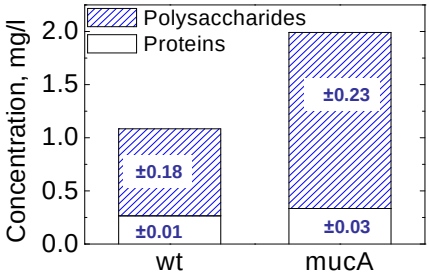
- EPS extracted from model strains biofilms (grown in 100mL batch LB culture for 36 hrs at 30^{o}C on 10g of 0.5 mm glass beads)
- EPS concentration was 0.9 mg/l as dissolved organic carbon
- EPS adherence characterization with quartz crystal microbalance with dissipation monitoring technology (QCM-D)
- Bacterial adhesion with parallel plate flow chamber

1) Mathee K., et al. Microbiology **1999**, *145,* 1349-1357.



Bar = 750 µm

EPS Characterization

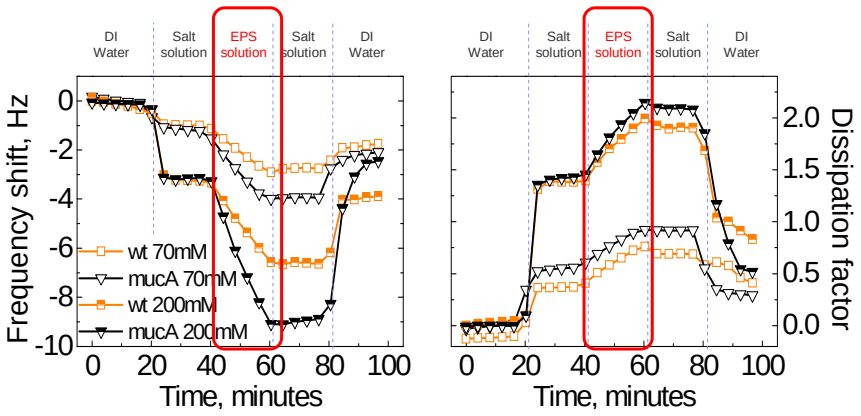


Polysaccharides[1] and proteins[2] content in 0.9 mg/L EPS[3,4] (as DOC) of the wt and mucA *P. aeruginosa* strains

Polysaccharides/proteins ratios in wt and mucA strains were 3.1 ±0.22 and 4.9 ±0.48, respectively.

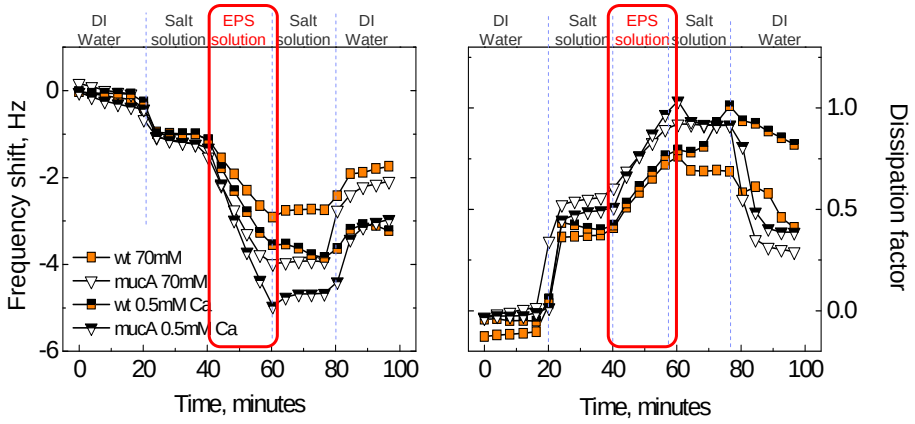
1) Dubois, M. et al. *Anal. Chem.* **1956**, *28*, 350-356; 2) Bradford, M. *Anal. Biochem.* **1977**, *72*, 248-254; 3) Herzberg, M. et al. *Environ. Sci. Technol.* **2009**, 43, 4393–4398; 4) Herzberg , M. et al. *Environ. Sci. Technol.* **2009,** 43, 7376–7383

EPS adherence in QCM-D – Ionic strength effect



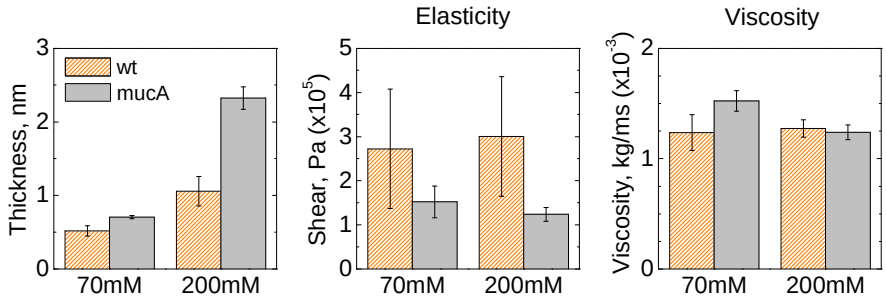
- Higher adsorption of EPS in 200mM NaCl compared to 70mM results in greater decrease in the sensor frequency and higher increase in dissipation factor
- A more efficient charge shielding of the EPS in a higher ionic strength increases EPS adherence.
- Alginate in the EPS (mucA) induces EPS adherence

EPS adherence in QCM-D – Calcium effect



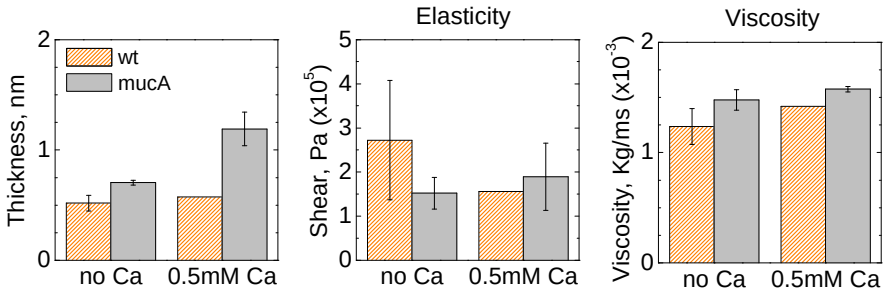
- Higher adsorption of EPS in the presence of calcium than in NaCl only
- EPS adsorption results in almost no change in dissipation factor – probably no change in viscoelasticity (dissipation due to adsorption not affected by calcium)

Modeling the effect of ionic strength on thickness, shear and viscosity values of the adsorbed EPS layers with Voigt based model - NaCl



- Ionic strength as well as presence of alginate are shown to increase the adsorbed layer thickness
- Mutant strain with more alginate has lower EPS elasticity (shear modulus)
- Wild type has a higher protein fraction in EPS layer and EPS layer is more elastic (may be due to additional positive charged amine functional groups
- Ionic strength shows no effect on the EPS layer viscosity

Modeling the effect of calcium on thickness, shear and viscosity values of the adsorbed EPS layers with Voigt based model – $CaCl_2$



- "0.5mM Ca" refers to system with 0.5mM Ca supplemented with NaCl to final IS of 70 mM; "no Ca" refers to 70mM NaCl
- Calcium is increasing only the thickness of the alginate rich EPS layer
- Calcium has almost no effect on the elasticity of the EPS layers but increase slightly EPS viscosity in both cases.
- Alginate is slightly increasing the EPS layer viscosity regardless the presence of calcium at this IS

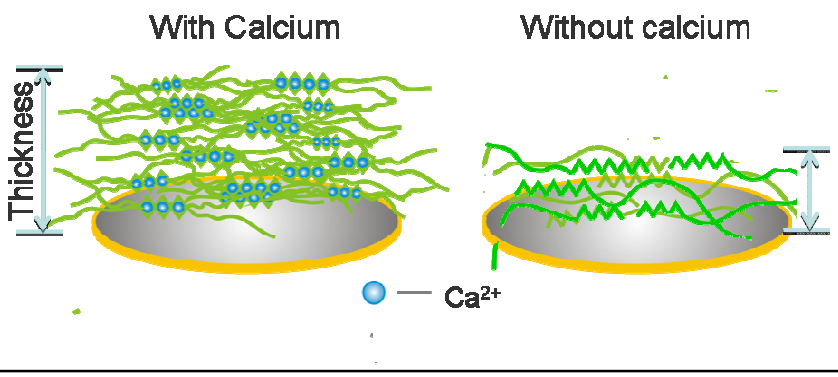
Proposed Mechanism

Increased IS – greater layer thickness

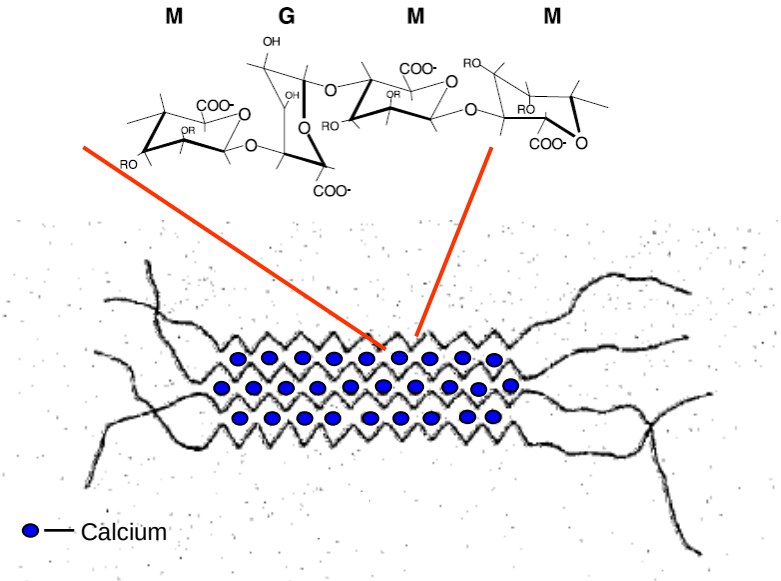
Increased alginate presence – greater layer thickness

Presence of calcium – greater EPS adsorption

- Due to calcium complexation with carboxylic groups in alginate and available surfaces
- Cross-linked "egg box" gel formation



Characteristic section of a monomer sequence in bacterial alginate
(**M:** mannuronate residues, **G:** guluronate residues)



1) Grant et al. FEBS letters (32) 1973, 195-198 ; 2) Lattner et al. Int. J. Biol. Macromolecules (33) 2003, 81-88

Parallel Plate Flow Cell

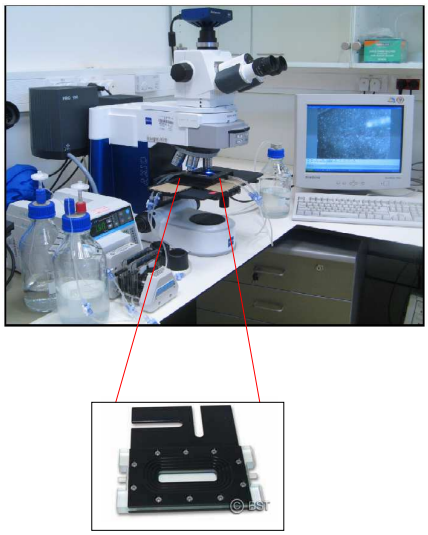
Flow cell: 47.5 long x12.7 wide x1.6 high (Biosurface technologies – Bozeman, Montana)

Flow rate: 2 ml/min, Pe=0.001

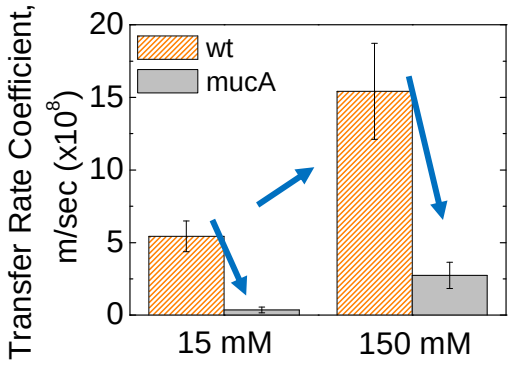
Shear stress on the surface: 6 sec^{-1}

Solution chemistry: 15, 150 mM NaCl solution

Strains: *Pseudomonas aeruginosa* PAO1 and its isogenic mucoid mutant PAO*mucA22*, PDO300

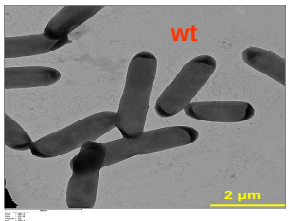


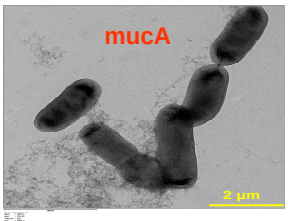
EPS effect on bacterial deposition



- Increased cell attachment with greater IS
- Over production of alginate reduced cell adherence – opposite of EPS trends in QCM
- Why?

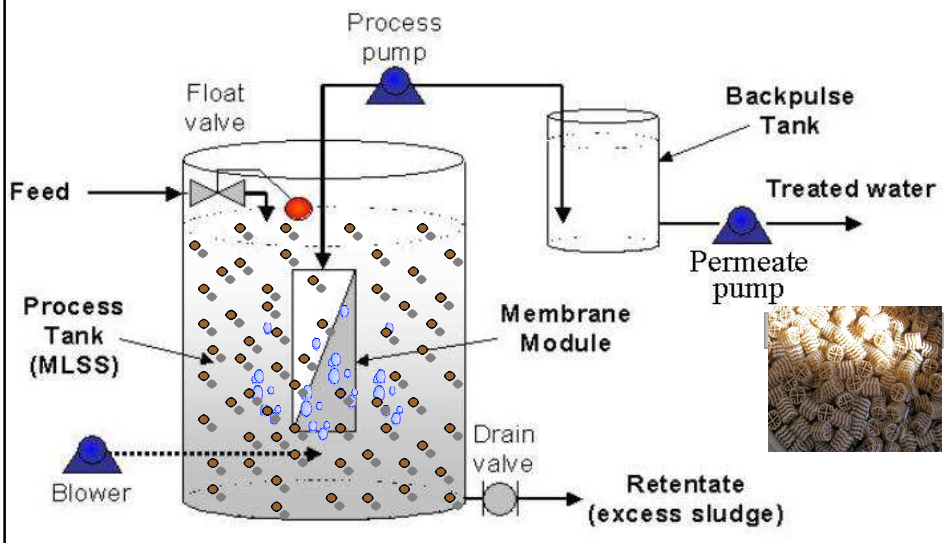
EPS effect on bacterial deposition





- TEM scans of wt and mucA cells show a clear presence of EPS. Suggests shielding of the mucA strain cells surface features (ie membrane bound features and pili) with the EPS matrix
- It seems that the exposed free appendages (Flagella) of the wt strain, enable better cell attachment to surfaces

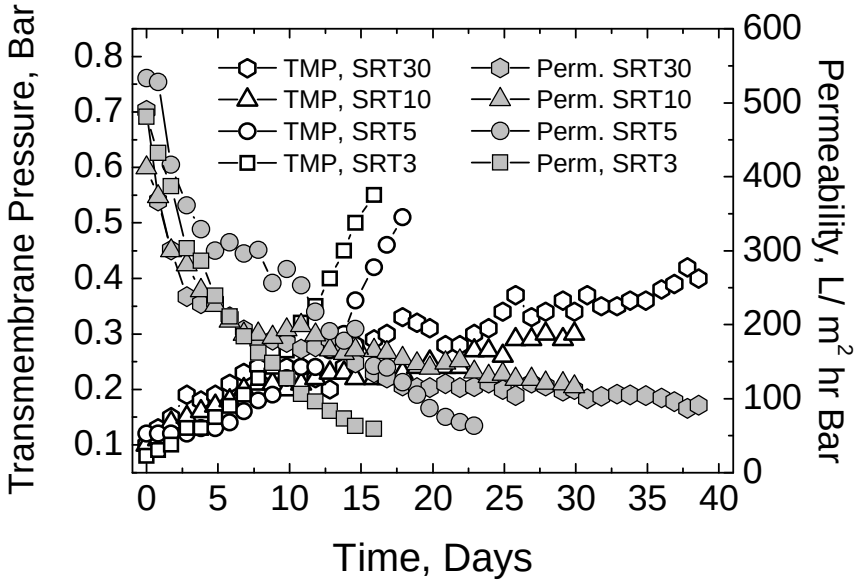
Case II: Operation of membrane bioreactor, EPS viscol-elastic properties and membrane fouling



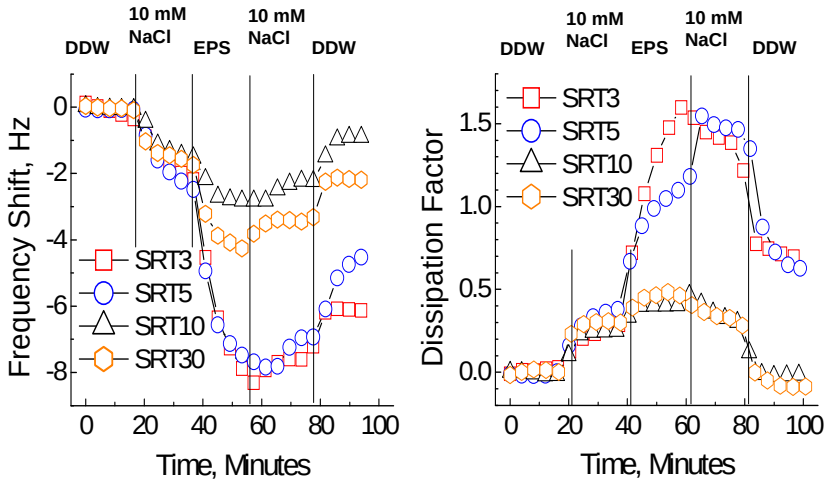
Operational conditions of the HG-MBR

Parameter	SRT=30 days	SRT=10 days	SRT=5 days	SRT=3 days
Temperature	15.5~29.4°C (mean 22.3°C)	22.2~28.9 °C (mean 26.3 °C)	20.0~30.6°C (mean 28.5 °C)	25.5~29.4°C (mean 28.0 °C)
Initial membrane permeability (L/(m^2.hr.bar)) at 20 °C	631.1	465.5	449.8	527.7
Initial membrane resistance (m^{-1})	0.56×10^{12}	0.76×10^{12}	0.79×10^{12}	0.67×10^{12}
Filtrate flux (L/(m^2.hr))	44.5 to 38.9	44.8 to 40.6	45.2 to 38.58	45.2 to 37.7
Aeration rate (m^3/hr)	2.3	2.3	2.3	2.3
Hydraulic retention time (hours)	5.1~5.8	5.0~5.5	5.0~5.9	5.0~6.0
pH in the reactor	6.6~7.8	6.2~6.9	6.6~6.9	6.7~7.1
Dissolved oxygen, mg O_2/L	0.31~6.5 (mean 2.6)	0.7~5.6 (mean 2.8)	0.25~3.66 (mean 0.89)	2.4~6.2 (mean 4.3)
MLSS range (mg/L)	2440 to 4580 (mean 4055)	1000 to 3520 (mean 2686)	1225 to 2110 (mean 1678)	1170 to 1575 (mean 1392)
AqWise carriers in the reactor	13.64 kg carriers in the reactor with a bulk filling ratio of 50%			
Operating time (days)	34	26	16	9

Trans-membrane pressure and permeability

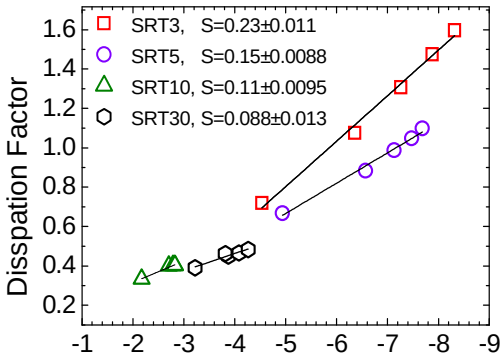


EPS adherence analysis



EPS viscoelasticity

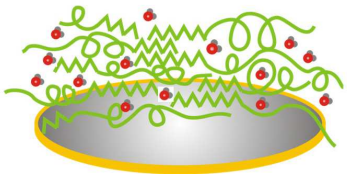
ΔD vs *ΔF:*



Voigt Model:

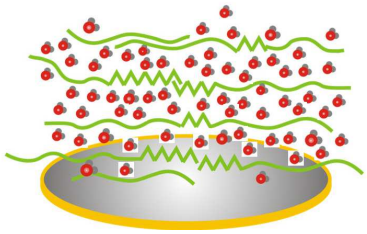
	SRT= 30 Days		10 Days		5 Days		3 Days	
Thickness, nm	2.8	2.4	3.5	2.6	2.2	2.8	3.3	2.8
Viscosity, $kg \cdot m^{-1} \cdot s^{-1}$	0.0018	0.0018	0.0015	0.0018	0.0011	0.0011	0.0010	0.0012
Shear modulus, Pa	$2.1 \cdot 10^5$	$4.5 \cdot 10^5$	$1.5 \cdot 10^5$	$1.5 \cdot 10^5$	$6.4 \cdot 10^4$	$4.5 \cdot 10^4$	$2.6 \cdot 10^4$	$3.2 \cdot 10^4$

Elastic and rigid EPS

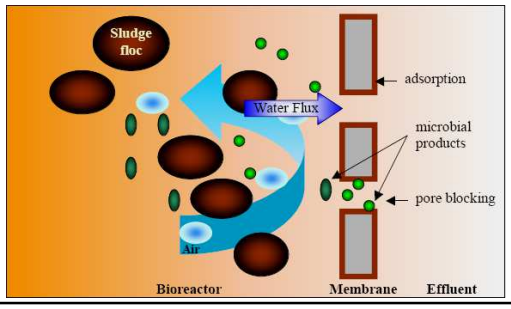


Less accessible to the membrane pores

Fluidic and highly hydrated EPS



More accessible to the membrane pores



Case III: Analysis, mechanisms, and applications of graft copolymerization with (+) and (–) monomers.

Generation of free radical in solution by the redox couple:

$$K_2S_2O_8 + K_2S_2O_5 \longrightarrow 4K^+ + SO_4^{\bullet -} + SO_4^{2-} + S_2O_5^{\bullet -}$$

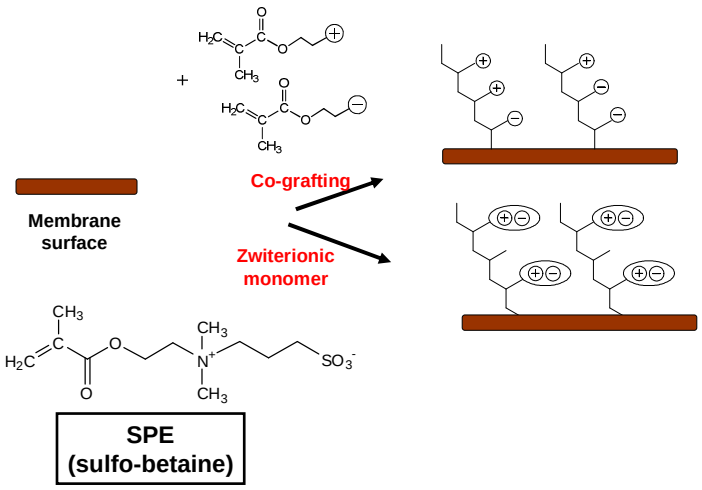
Potassium persulfate

Potassium metabisulfite

Bamford and K. Al-Lamee, Polymer (1994)

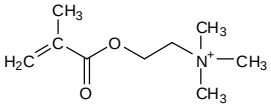
- **Grafting occurs on the surface as well as in solution**

Our approach:

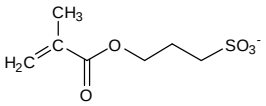


Objectives

1. **Anti-fouling of PVDF UF membranes having increased wettability with net zero surface-charge**
2. **Surface characterization, swelling, reduced adsorption of extracellular polymeric substances (EPS) to the modified surface and eventually reduced membrane fouling**

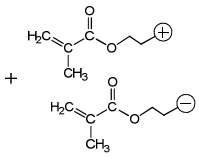


MOETMA
Methacryloyloxy ethyl trimethylammonium

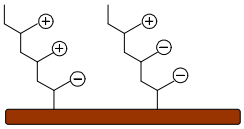


SPM
Sulfopropyl methacrylate

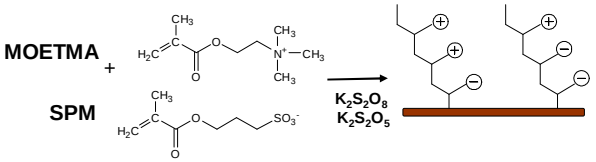
Membrane surface



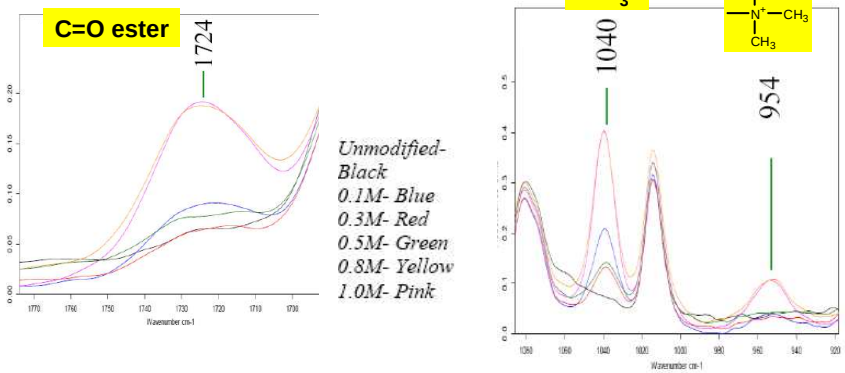
Co-graft-polymerization



MOETMA + SPM modification

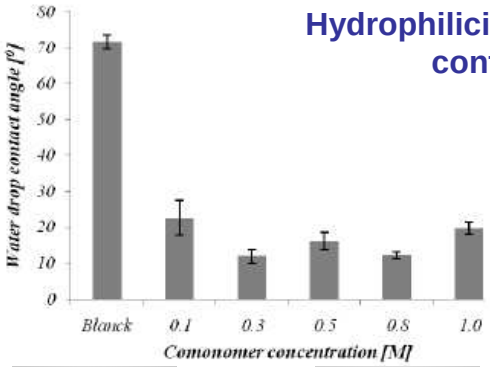


ATR-FTIR spectrum:

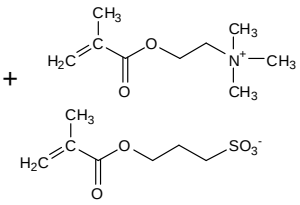


Achieve high grafting yields of sulfo and ammonium !

Hydrophilicity – by water drop contact angle



+

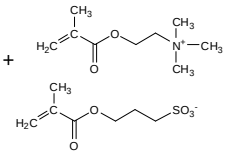


MOETMA + SPM

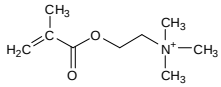
Achieve dramatic increase in hydrophilicity (wettability) in all concentrations used!!

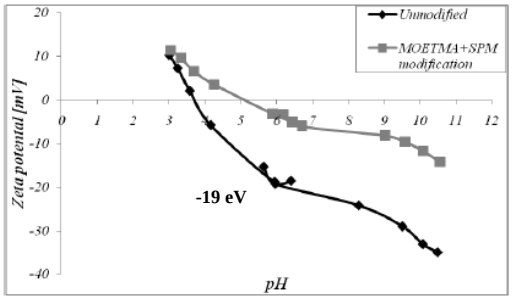
Zeta-potential of RO membrane surface after grafting

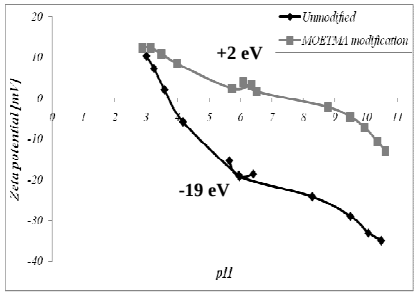
MOETMA + SPM +



MOETMA only



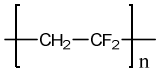




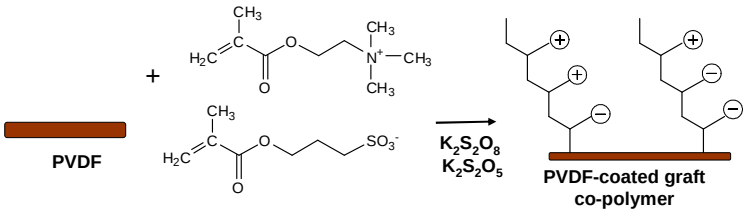
Graft Copolymerization On PVDF-coated surface using SPM + MOETMA

Polyvinylidene fluoride - PVDF

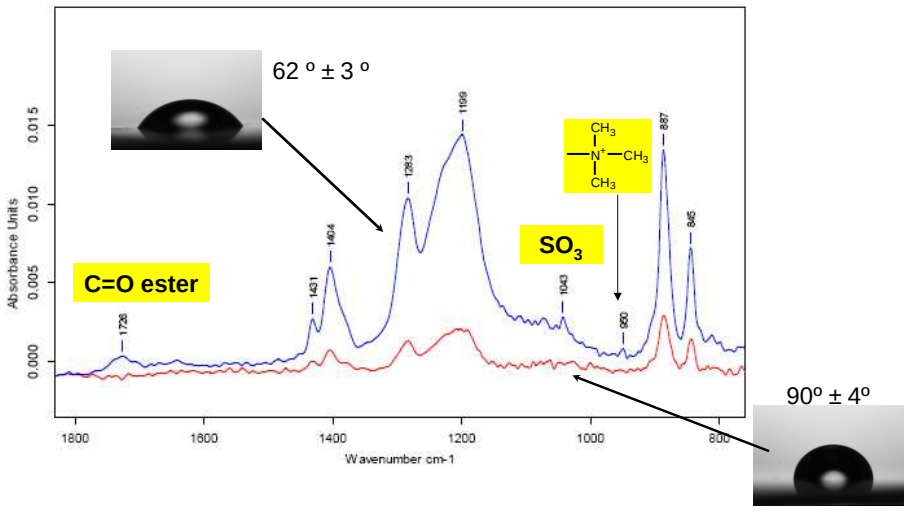
- ✓ **Ultrafiltration membranes**
- ✓ **Hydrophobic polymer**



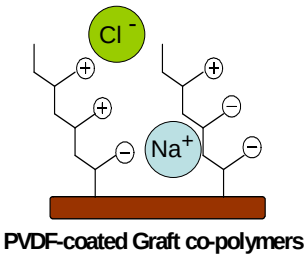
1) Surface characterization of the grafted PVDF surface
2) Antibiofouling properties and involved mechanisms



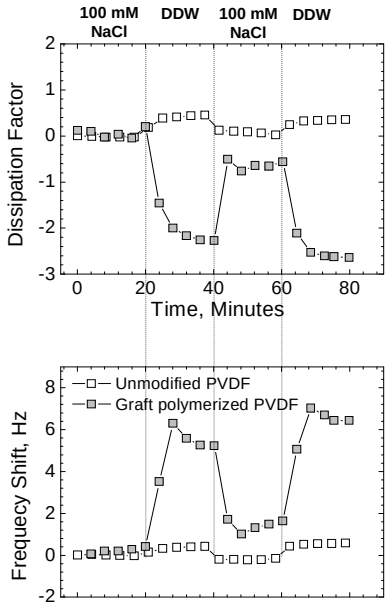
ATR-FTIR and water drop contact angle of PVDF-surface grafted with MOETMA and SPM



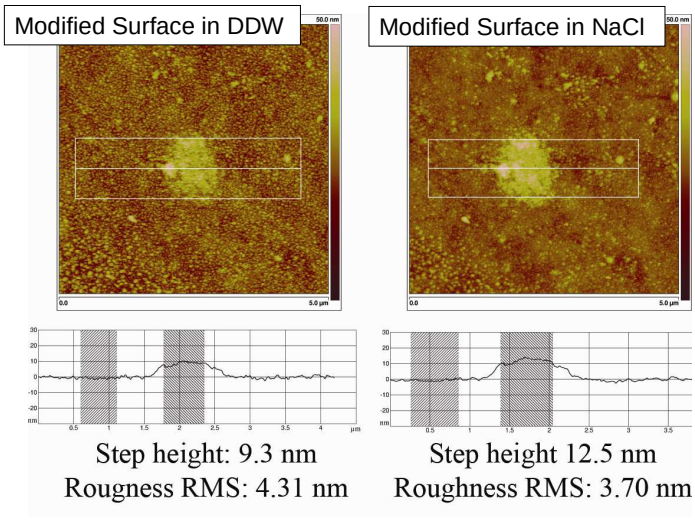
Characterizing swelling with QCM-D



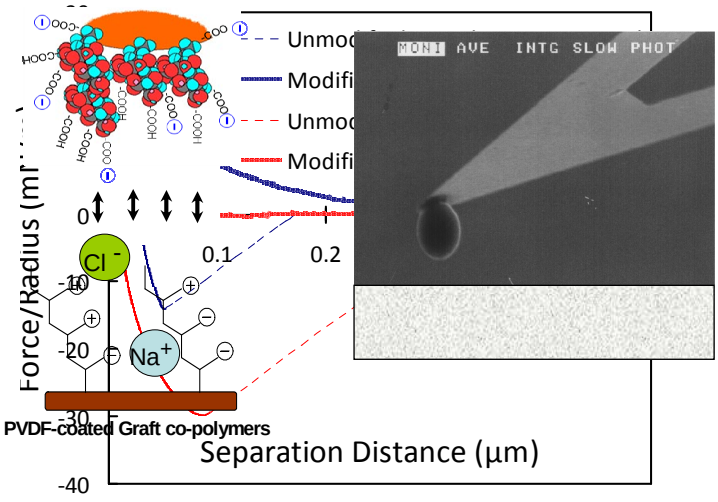
PVDF-coated Graft co-polymers



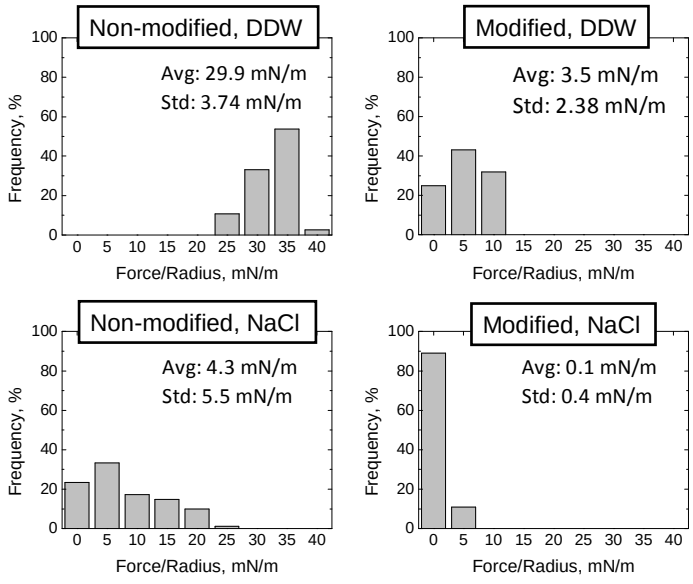
Characterizing swelling with atomic force microscopy



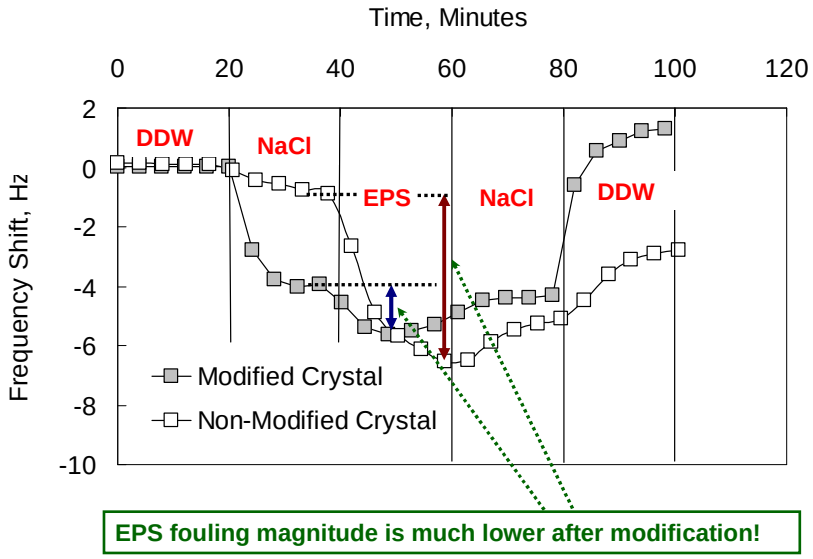
How swelling affects force interactions with model foulant?



Force interactions with model foulant

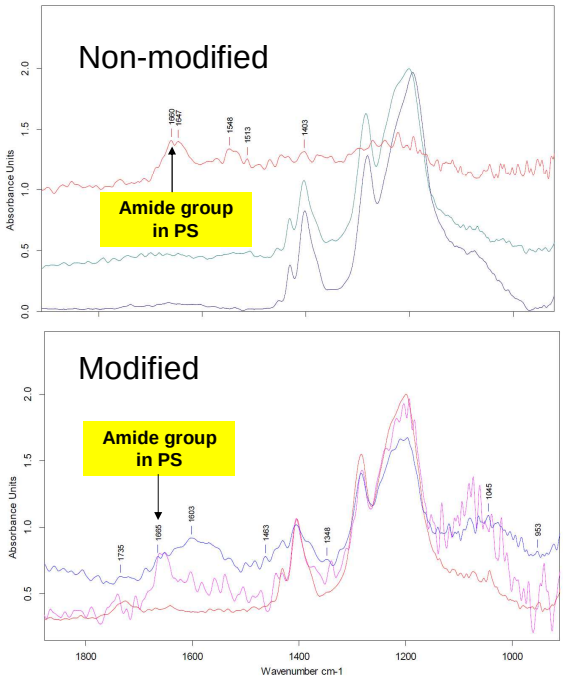


Effect of surface modification on adhesion of EPS



EPS fouling magnitude is much lower after modification!

ATR-FTIR spectrum of fouled QCM-D sensors with EPS



Concluding Remarks

- We define how aquatic conditions as well as polysaccharides content affect EPS visocoelastic and adherence properties.
- We correlate between the operational conditions of membrane bioreactors (MBRs), EPS produced and their propensity to foul the UF membrane.
- We study the interactions between MBR EPS and zwitterionic grafted polymer brushes, where we elucidate the mechanisms involved in a drastic reduced EPS adhesion.

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- **Wang Ying**
- **Jenia Gutman**

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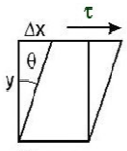
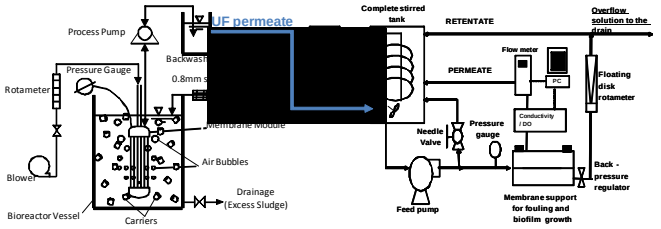
Background - Viscoelasticity of Biopolymer Layers



$$G = \frac{\tau}{\gamma} \qquad \frac{\Delta x}{y} = \tan\theta = \gamma = measured\ strain$$

- Viscoelastic materials possess complex shear modulus: $G^* = G' + iG''$, which measures the viscous (G'', loss modulus) and elastic (G', storage modulus) moduli when exposed to an oscillatory shear strain of frequency ω
- Purely elastic materials (like a crystalline solid) G'' = 0
- Purely viscous fluid G'=0
- In more complex fluids such as polymers (and biofilms) G', G''≠0, due to their viscoelastic properties
- Biofilms mechanical behavior has the characteristics of a viscoelastic solid with a high storage modulus G' and a relatively small loss modulus G''


Process Pump
UF permeate
Complete stirred tank
RETENTATE
Overflow solution to the drain
Backwash
Flow meter
PERMEATE
Pressure Gauge
Rotameter
0.8mm s
Membrane Module
Floating disk rotameter
Conductivity / DO
Needle Valve
Pressure gauge
Blower
Air Bubbles
Back - pressure regulator
Drainage (Excess Sludge)
Feed pump
Membrane support for fouling and biofilm growth
Bioreactor Vessel
Carriers