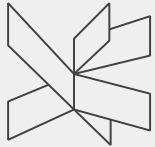


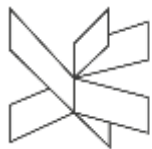
Gør tanke til handling  
VIA University College



# Mikrobiel vækst i plastrør - udfordringer og muligheder

Ditte A. Søborg – biofilmens sammensætning gennem 1,5 år i PE-rør  
Torben L. Skovhus – idriftsættelse af PE-rør i drikkevandssystemet

Research Centre for Built Environment, Energy, Water and Climate  
VIA University College, Horsens, Denmark

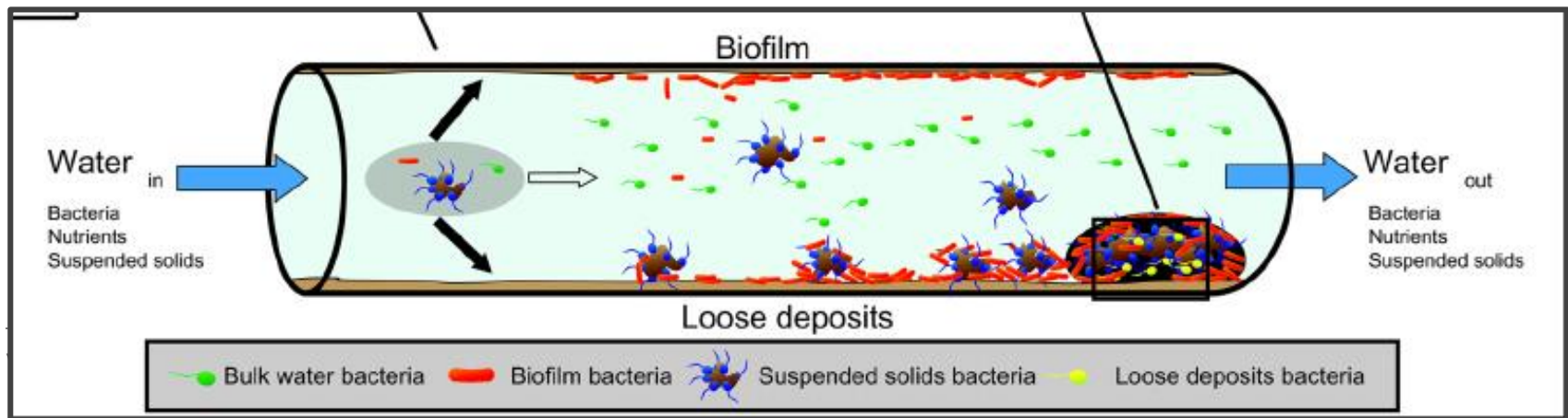
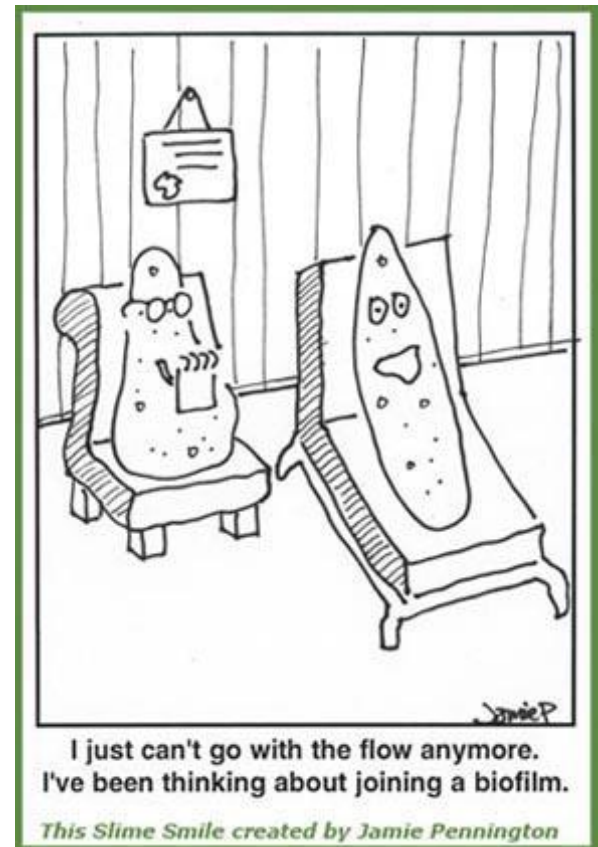


Bring ideas to life  
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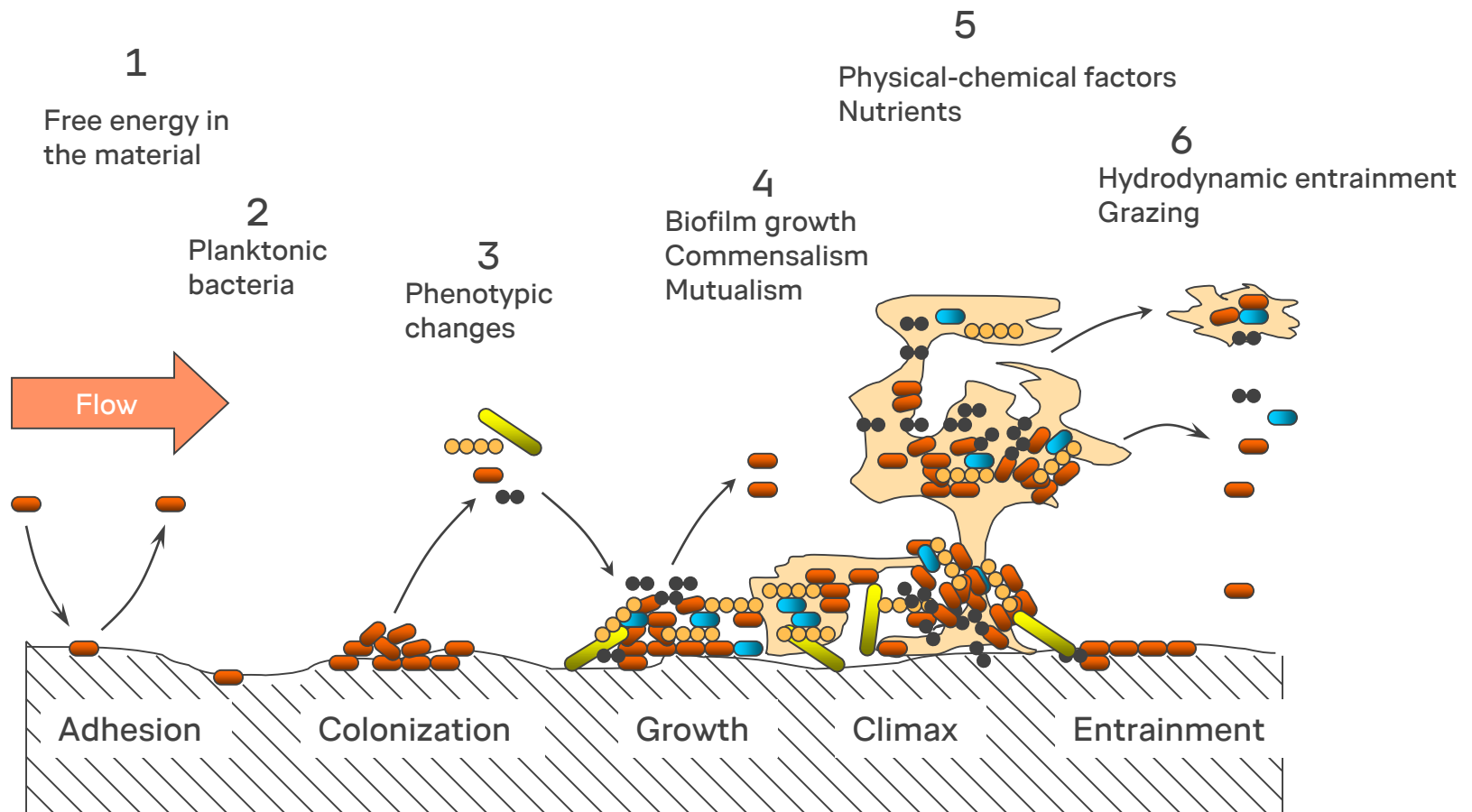


# Biofilm

- Den mest succesfulde form for liv
- 80-95 % af mikroorganismer i drikkevandssystemet sidder bundet i biofilm, sediment og til partikler

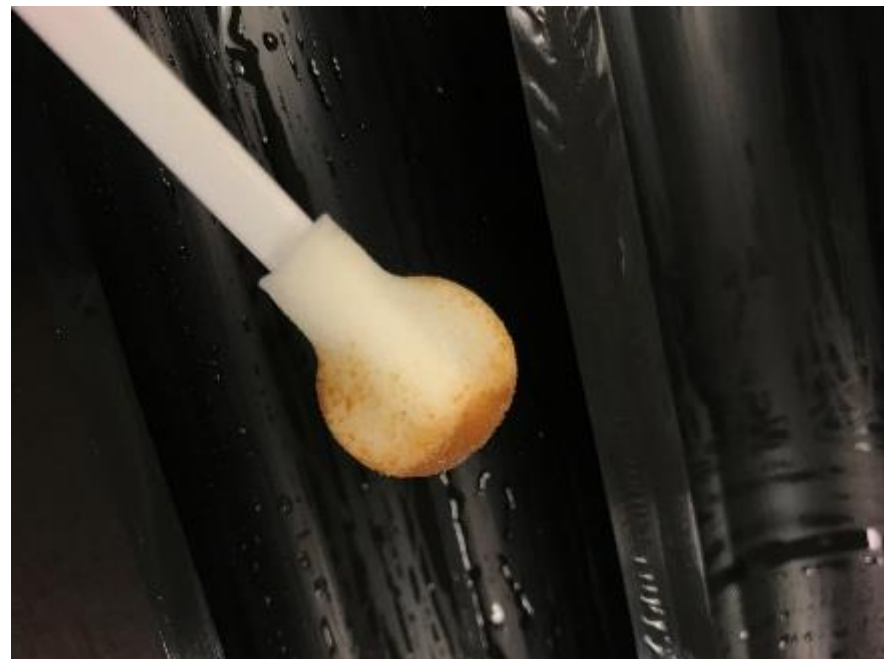
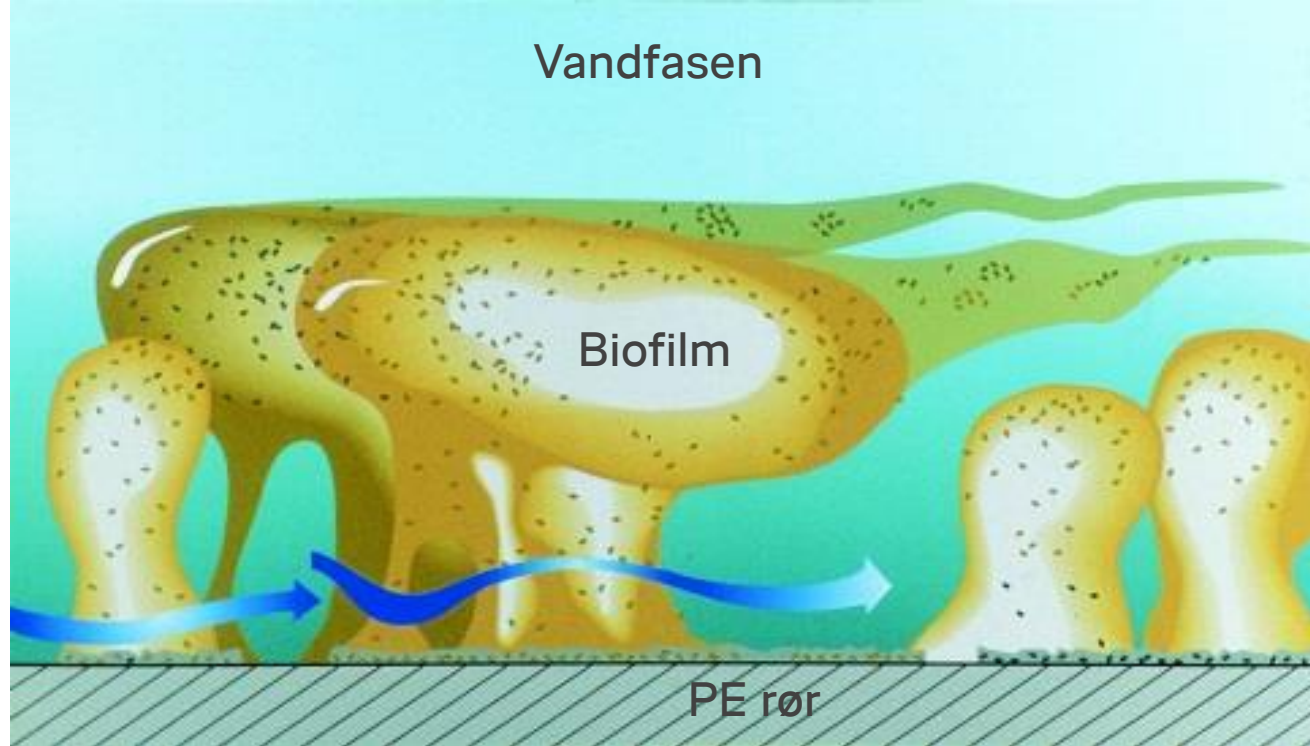


# Udvikling af biofilm på PE rør



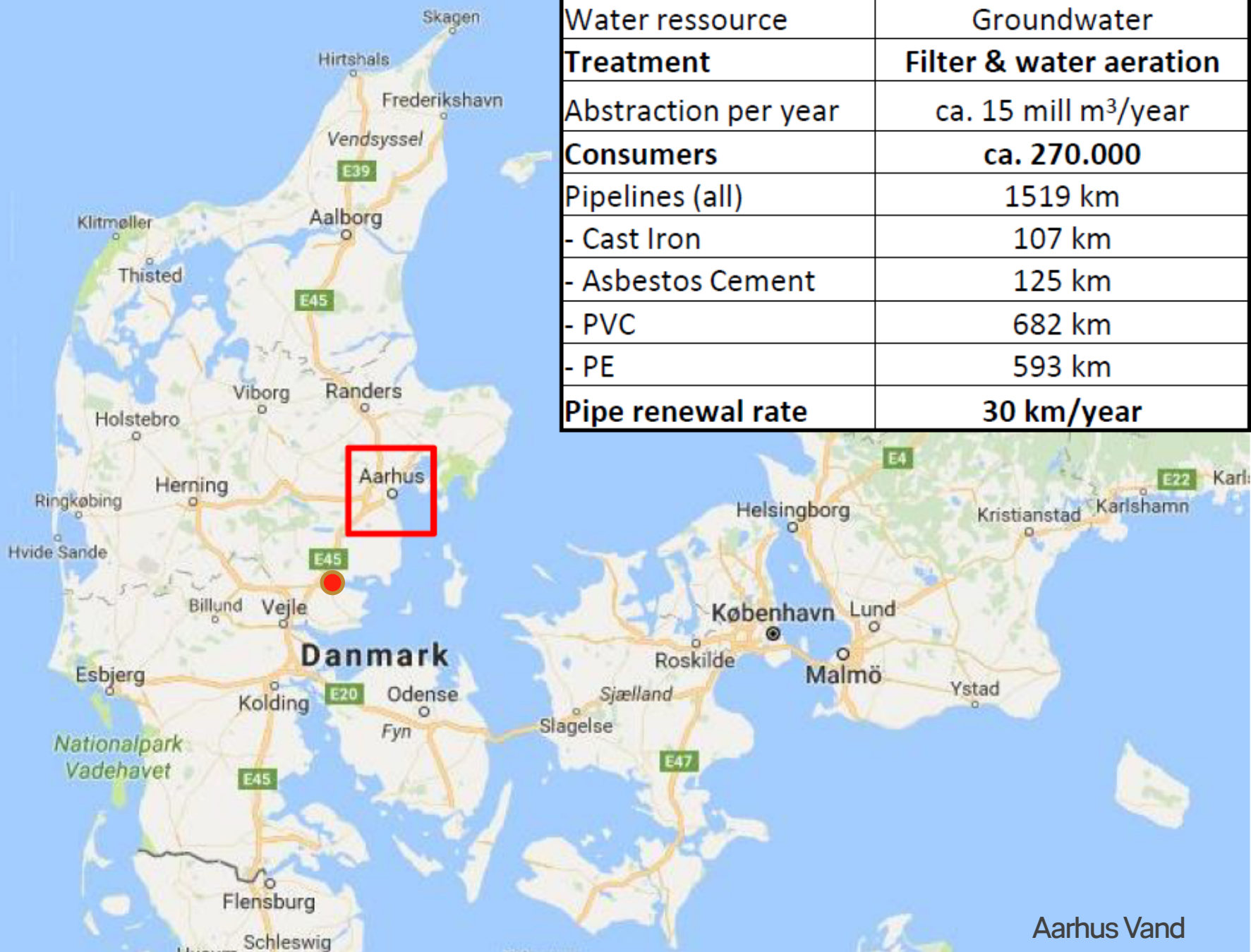
Idea from Paul Stoodley et al.

# Biofilm i PE rør



## Facts about Aarhus Water

Water ressource	Groundwater
Treatment	Filter & water aeration
Abstraction per year	ca. 15 mill m <sup>3</sup> /year
Consumers	ca. 270.000
Pipelines (all)	1519 km
- Cast Iron	107 km
- Asbestos Cement	125 km
- PVC	682 km
- PE	593 km
Pipe renewal rate	30 km/year



# Arbejdspakker

1. Opstart/state-of-the-art
2. Kortlægning af biofilm felten
3. Etablering af biofilm i testrigs
4. Opstilling af Biofilmsensor i felten
5. Afrapportering til VTU-Fonden



## Characterization of young and mature biofilms in the Danish drinking water distribution system

Ditte A. Søborg<sup>1</sup>, TL Skovhus<sup>1</sup>, JO Andreasen<sup>2</sup>, KB Kristensen<sup>2</sup>, KL Hansen<sup>2</sup>, and B Højris<sup>3</sup>

<sup>1</sup>Research Group for Energy and Environment, VIA University College, Denmark  
<sup>2</sup>Aarhus Water A/S, Denmark, <sup>3</sup>GRUNDFOS Holding A/S, Denmark

**NDWS 2019**

### BACKGROUND

Biofilm is considered beneficial in the non-chlorinated Danish drinking water distribution system, as it increases the microbiological stability of the water.

When introducing new pipe sections in the distribution network, a biofilm develops on the pipe wall. There is limited knowledge on the influence of water quality, pipe material, the existing biofilm upstream the new pipe section, flow velocity, etc. on this young biofilm.

The influence of biofilms on the water quality in the long-term during the commissioning of new pipe sections remains poorly understood.

This project aims to analyze the long-term effect of the developed biofilm in newly installed drinking water PE pipes on the water quality to be able to optimize the commissioning procedure for installing new pipe sections in an existing pipe network.

Two different sampling locations (installed biofilm rigs) were investigated for biofilm development. One biofilm rig was installed at a waterworks close to the ground water source (TBR) and another biofilm rig in a water storage facility near the consumer (BUS).

### METHODS




Figure 1. Biofilm rig and sampling of biofilm. (A) The biofilm rig at a waterworks (TBR) with instrumentation (flowmeter, thermometer, CR-NDP05 BACMON). Length approx. 50 m. (B) PE pipe cut out for analysis of ATP, DAPI, qPCR, and NGS. (C) The same PE pipe cut in half in the lab and swabbed for biofilm. A similar setup and procedure was followed for the biofilm rig placed in a water storage facility (BUS).

Water was sampled from the inlet and outlet of the rigs and tested for HPC, Coliact, ATP, and DAPI. Water and biofilm was sampled approx. every 1.5 months during 1.5 years.

### BIOFILMS IN PE-PIPES ARE SHAPED BY THE LOCATION IN THE DISTRIBUTION SYSTEM

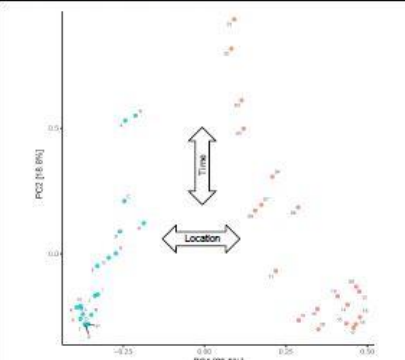


Figure 2. Principal-component analysis based on 16S rRNA gene sequencing of biofilm from test rigs TBR (1-10) and BUS (A-T). Each point represents the full diversity in a sample at a given time from one of the two locations. Sample 1+2 (and A+B) are true replicates (and so on). After 1.5 years, samples from each location clustered separately along PC1, suggesting that the PC1 axis explains variations based on location (effect of upstream factors). Samples distributed along the PC2 axis in relation to the time of sampling (maturation of the biofilm).

### YOUNG AND MATURE BIOFILMS DIFFER IN MICROBIAL COMMUNITY COMPOSITION




Figure 3. Heatmap showing the 50 most abundant genera in biofilms from test rigs TBR (1-10) and BUS (A-T). Young biofilms from both locations was dominated by genera of Comamonadaceae and Caulobacteraceae. The community composition of mature biofilms differed. A genus of Saccaribacteres was the most dominant in the mature biofilm of TBR (accounting for ~40% in samples 11-20, from 10 months to 1.5 years). Mature biofilm of BUS (samples 1-T from 8 months to 1.5 years) was dominated by genera of Saccaribacteres (1-15%) and the genus *Sphaerotilus* (7-10%). A high presence of the Actinobacteria family TM146 was observed at both locations, especially in samples of 3 to 10 months old biofilm.

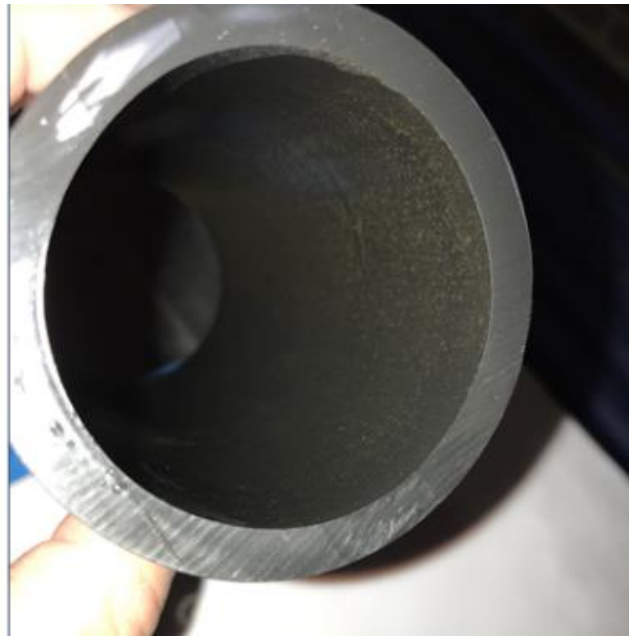
### CONCLUSIONS

Young biofilm from two different locations in the Danish drinking water distribution system was dominated by genera of Comamonadaceae and Caulobacteraceae.

A mature biofilm was considered reached when a stable community composition was observed. As seen from the PCA plot in Figure 2, the samples 1-T and 11-20 clustered together, which showed that a mature biofilm was reached at BUS after 8 months and at TBR after 10 months. The microbial community composition of the mature biofilm differed between the two locations as seen from the heatmap in Figure 3. The diversity of the mature biofilm was higher in BUS than TBR, as seen from a Shannon index of approx. 5 compared to approx. 3. This difference is most likely related to upstream factors such as water quality, pipe material, the existing biofilm upstream the new pipe section, flow velocity, etc.

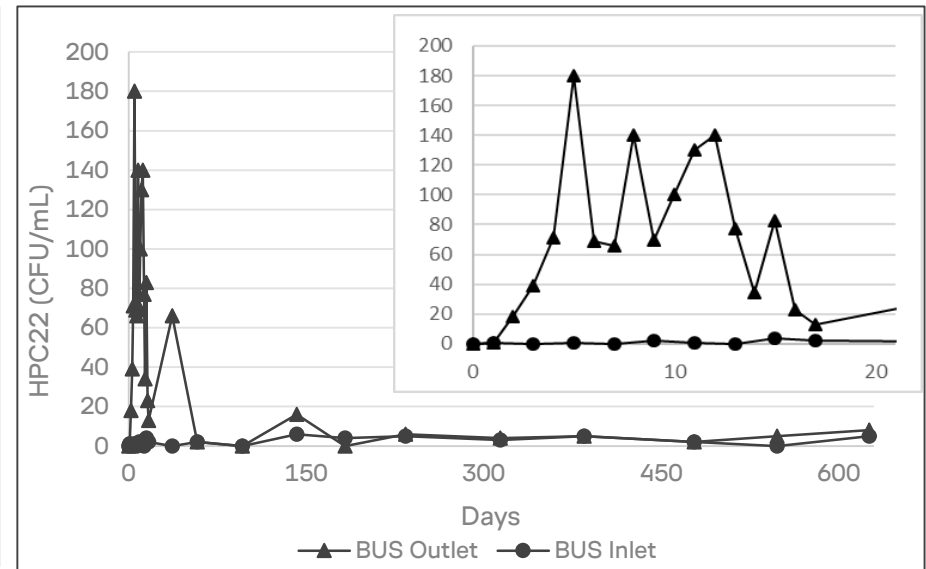
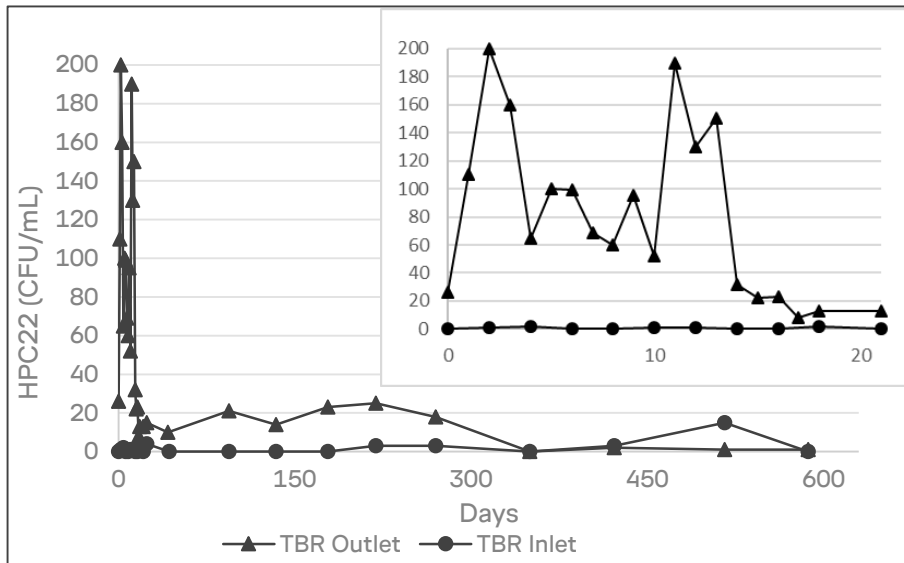
Results of water samples showed the importance of reaching a mature biofilm for the biological stability of the water. There was a clear decrease in HPC, ATP and DAPI counts at the time when the microbiological diversity of biofilms reached a steady state at both locations. Coliact results were at all times meeting national drinking water requirements (<1 Coliform/E.col per 100 mL).

# Langtidsudvikling af biofilm i test-rigs



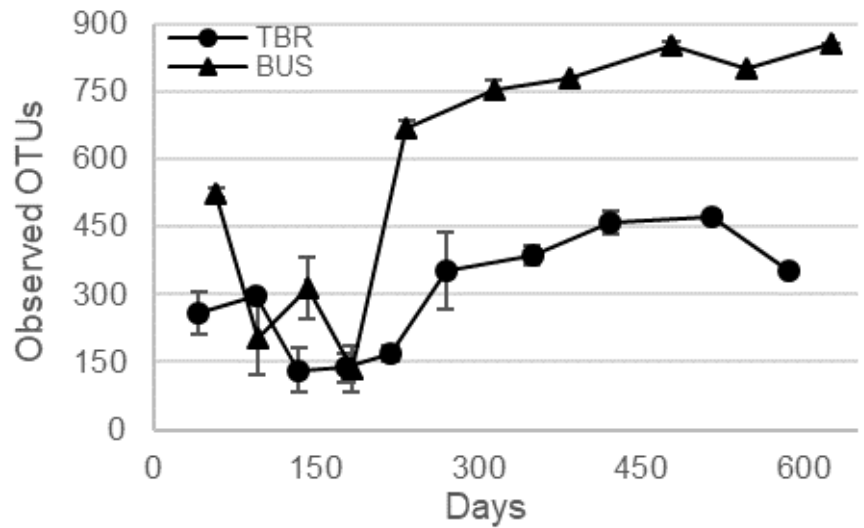
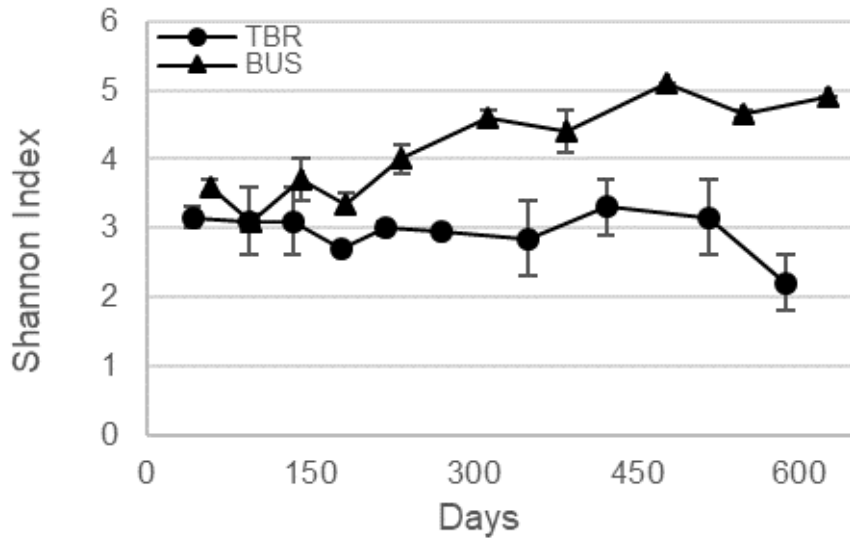
# Resultater af vandprøver fra test rigs

- Vandtemp.: 7,7-11,8 °C
- Organisk stof: 1,5 mg/L
- Mikrobiologi: < 1 coliforme og < 1 *E. coli*/100 mL
- Kimtal ved 22°C: Observation af toppe, ellers <20 CFU/mL

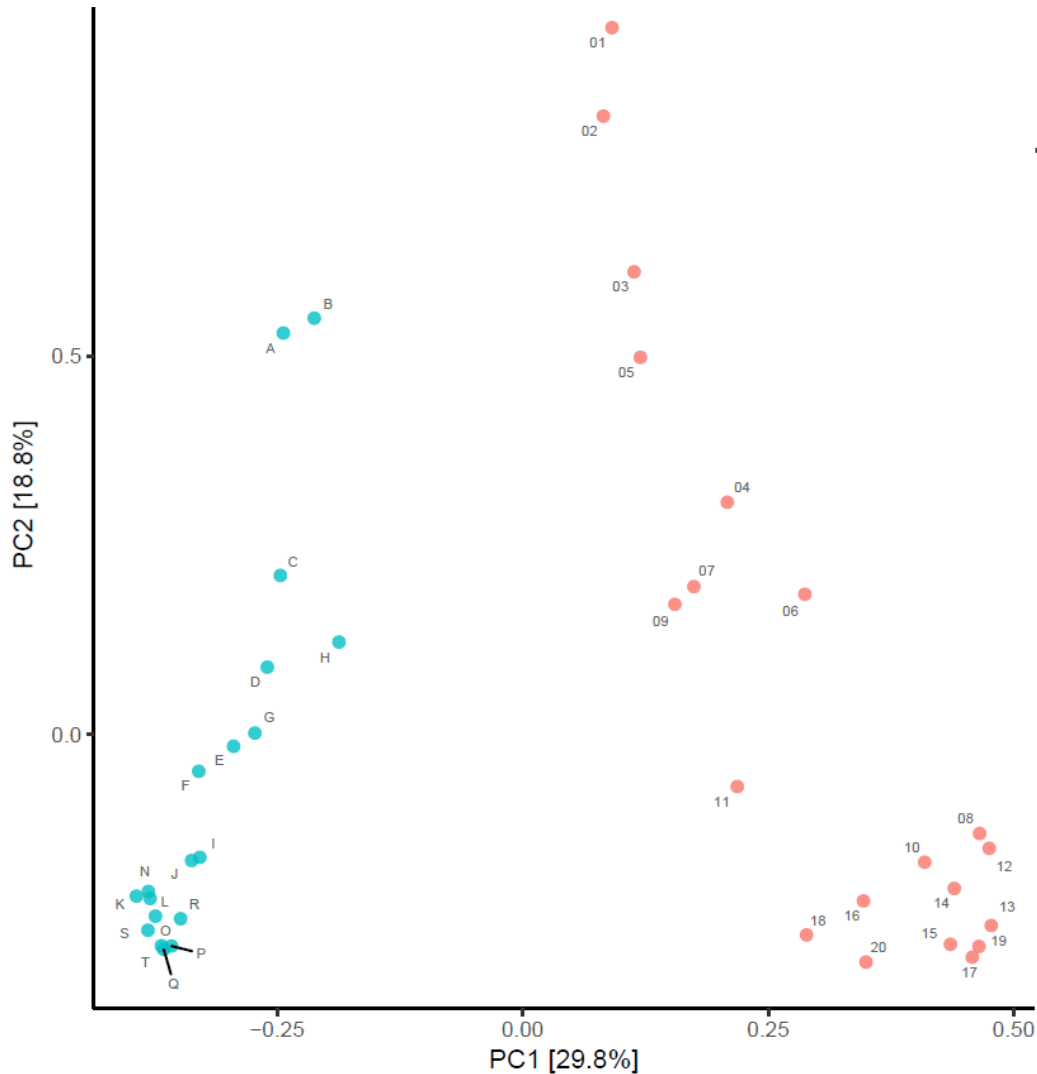




# Diversitet af biofilm i test rigs



# Diversitet af biofilm i test rigs



**Principal component analysis**

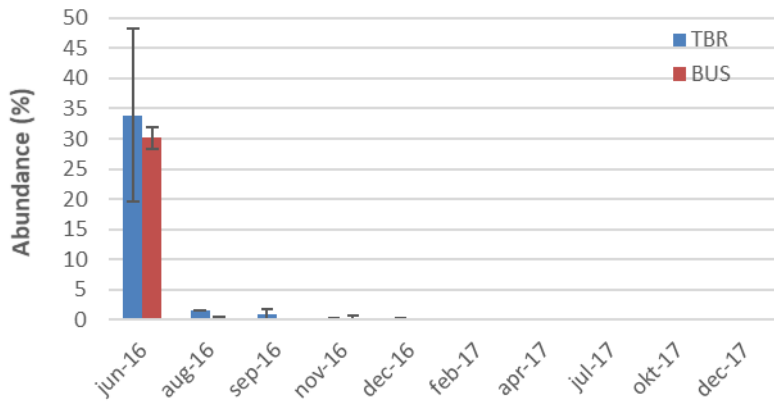
TBR (1-20)

BUS (A-T)

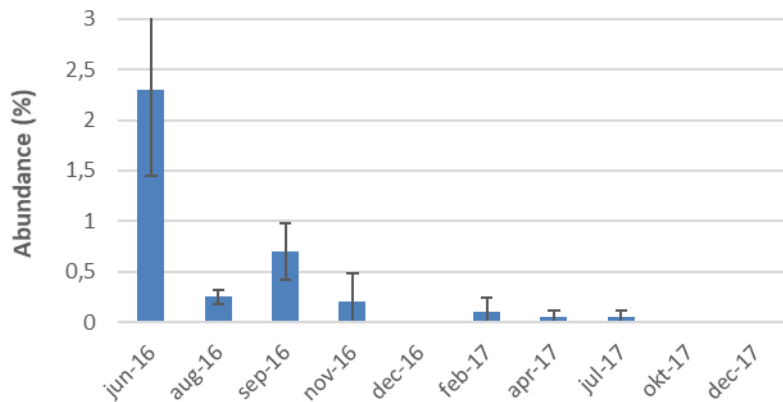
Moden biofilm efter ca. 8-9 mdr.

# Tidlige biofilmmaktører/PE aktører

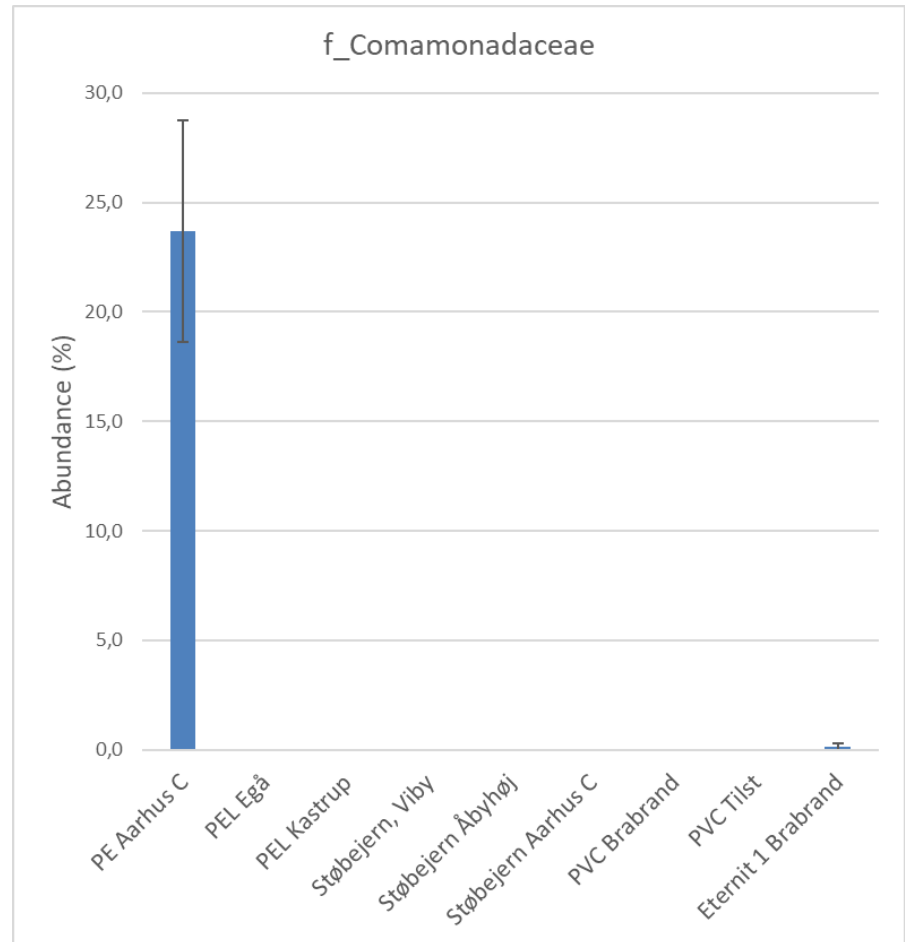
f\_Comamonadaceae



f\_Caulobacteraceae



f\_Comamonadaceae



# Konklusioner

1. Det tager mere end et halv år før en moden biofilm er dannet i nye PE-rør
2. Tidlige biofilmaktører i PE-rør omfatter muligvis familierne *Caulobacteraceae* og *Comamonadaceae*
3. Opstrøms faktorer påvirker biofilmsammensætningen
4. Rørmaterialet påvirker evt. biofilmsammensætningen

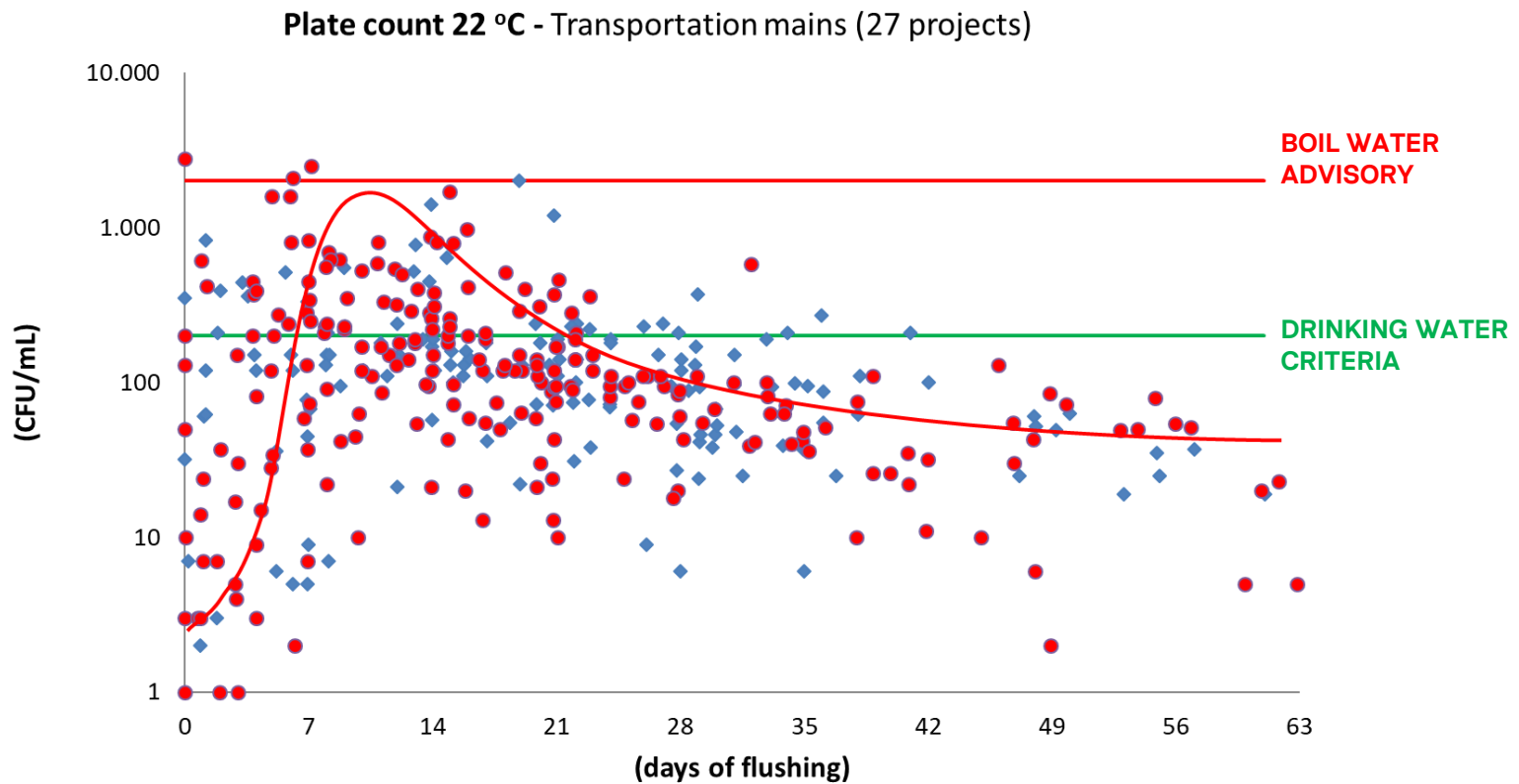
# Idriftsættelse af nye PE rør



1. Svampning af nyt rør
2. Skylning med 4x nyt rørs rørvolumen
3. Tæthedsprøvning



# Problemer med forhøjet Kimtal 22 hos Aarhus Vand i 2015



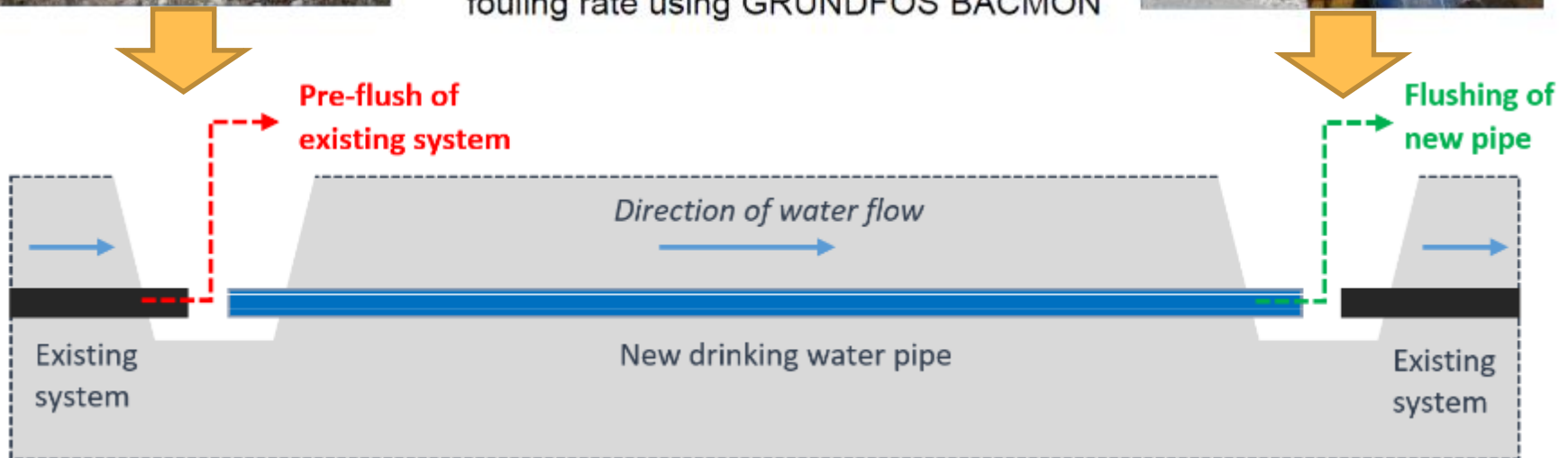
# Problemstillinger i arbejdspakken

1. Stort forbrug af mikrobiologiske analyser, mandetimer og vand ved idriftsættelse af nye PE rør (ledningsanlæg)
2. Er de observerede "kimtals-toppe" et udbredt fænomen?
3. Er de observerede "kimtals-toppe" et reelt problem for vandkvaliteten?
4. Optimering af idriftsættelsesproceduren for nye PE rør (planlagte idriftsættelser af nye ledningsanlæg)
5. I hvilken grad kan GRUNDFOS BACMON (online bakteriesensor) bidrage til optimeret idriftsættelse?

# EXPERIMENTAL SETUP



- Dimensions of PE pipe: 500m, Ø30cm, 470m<sup>2</sup>
- Flushing: 1 day pre-flushing, 41 days of flushing
- Drinking water criteria: HPC22 < 200 CFU/ml
- All water was discharged to the sewer system during flushing
- Analysis of HPC22/37, ATP, qPCR, Sequencing and biotic/abiotic particles and fouling rate using GRUNDFOS BACMON





202 Lystrupvej

Lystrup, Midtjylland

Google, Inc.

Street View - sep. 2017



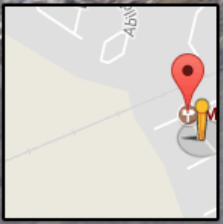
Google

Mosevangen

Lystrup, Midtjylland

Google, Inc.

Street View - sep. 2017



Google

# mobil BACMON

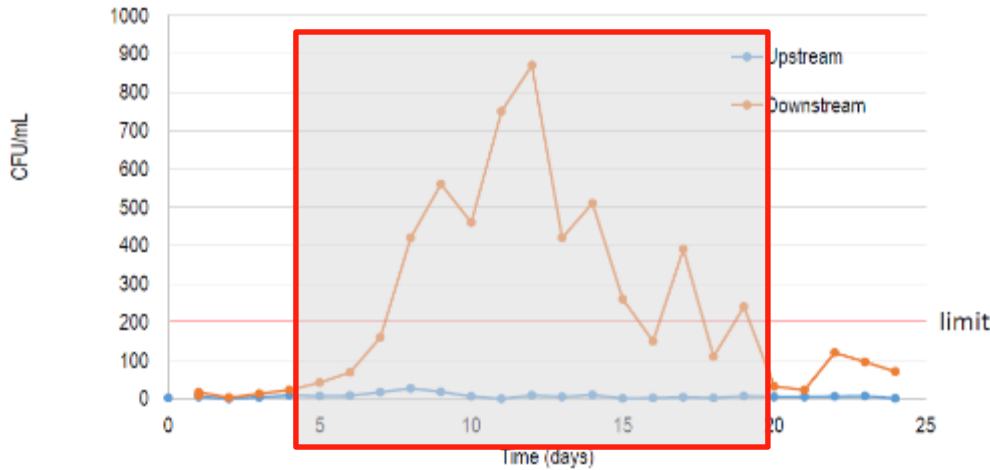


# Resultater - vandprøver

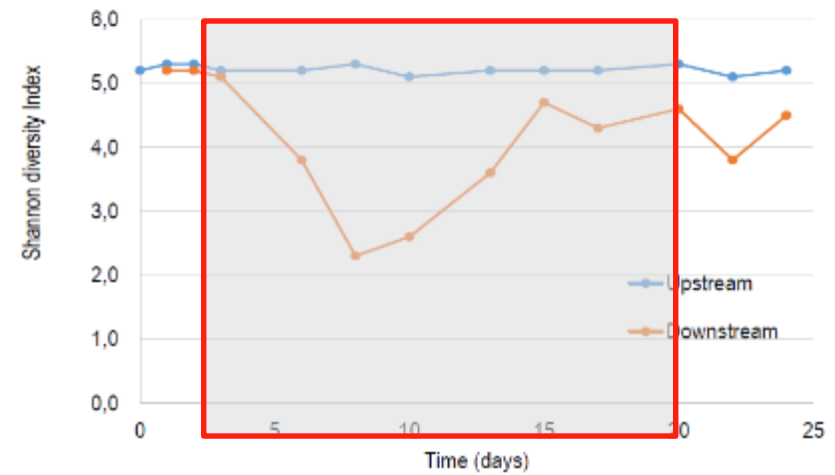


# Mikrobiologiske resultater samlet

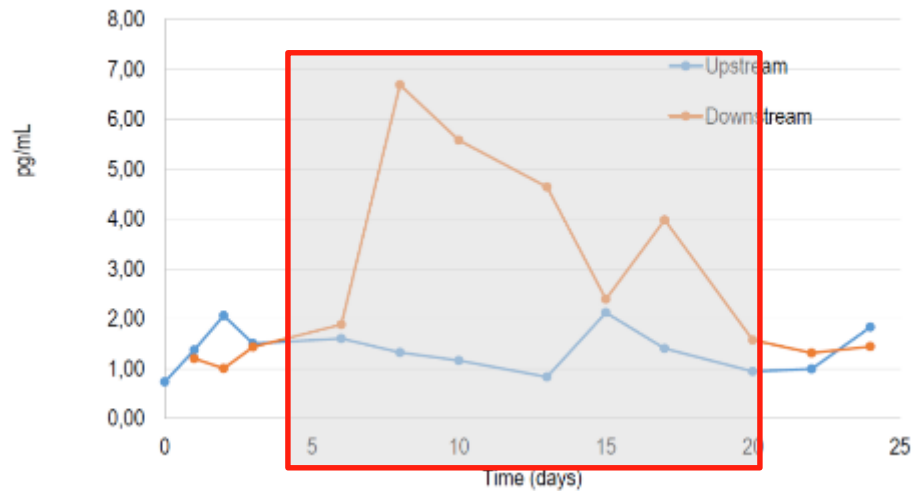
## HPC 22°C



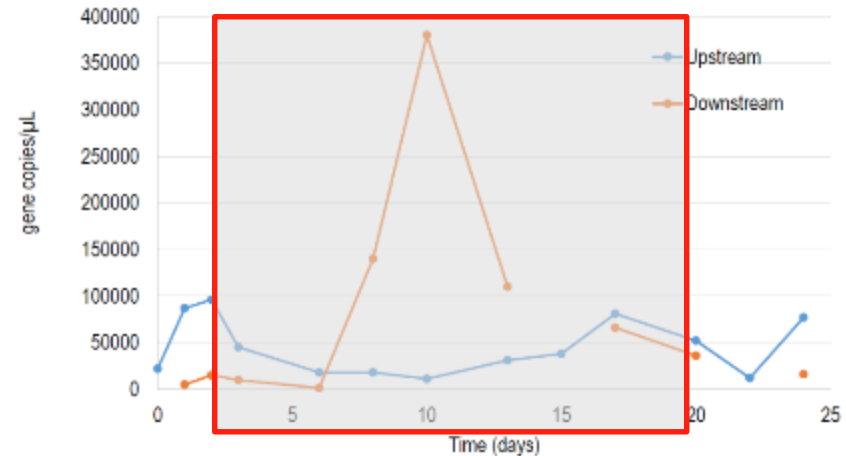
## Diversity



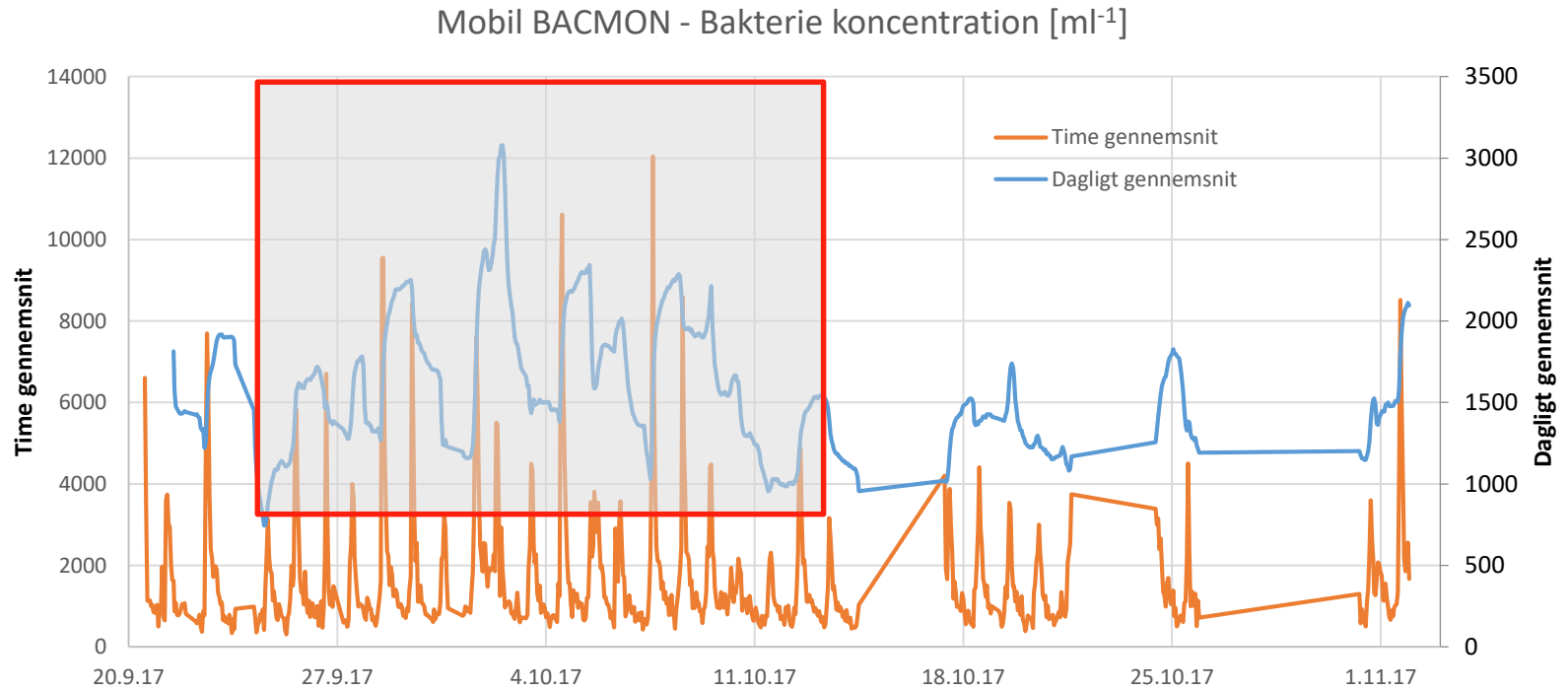
## ATP



## qPCR



# BACMON - bakteriekoncentration

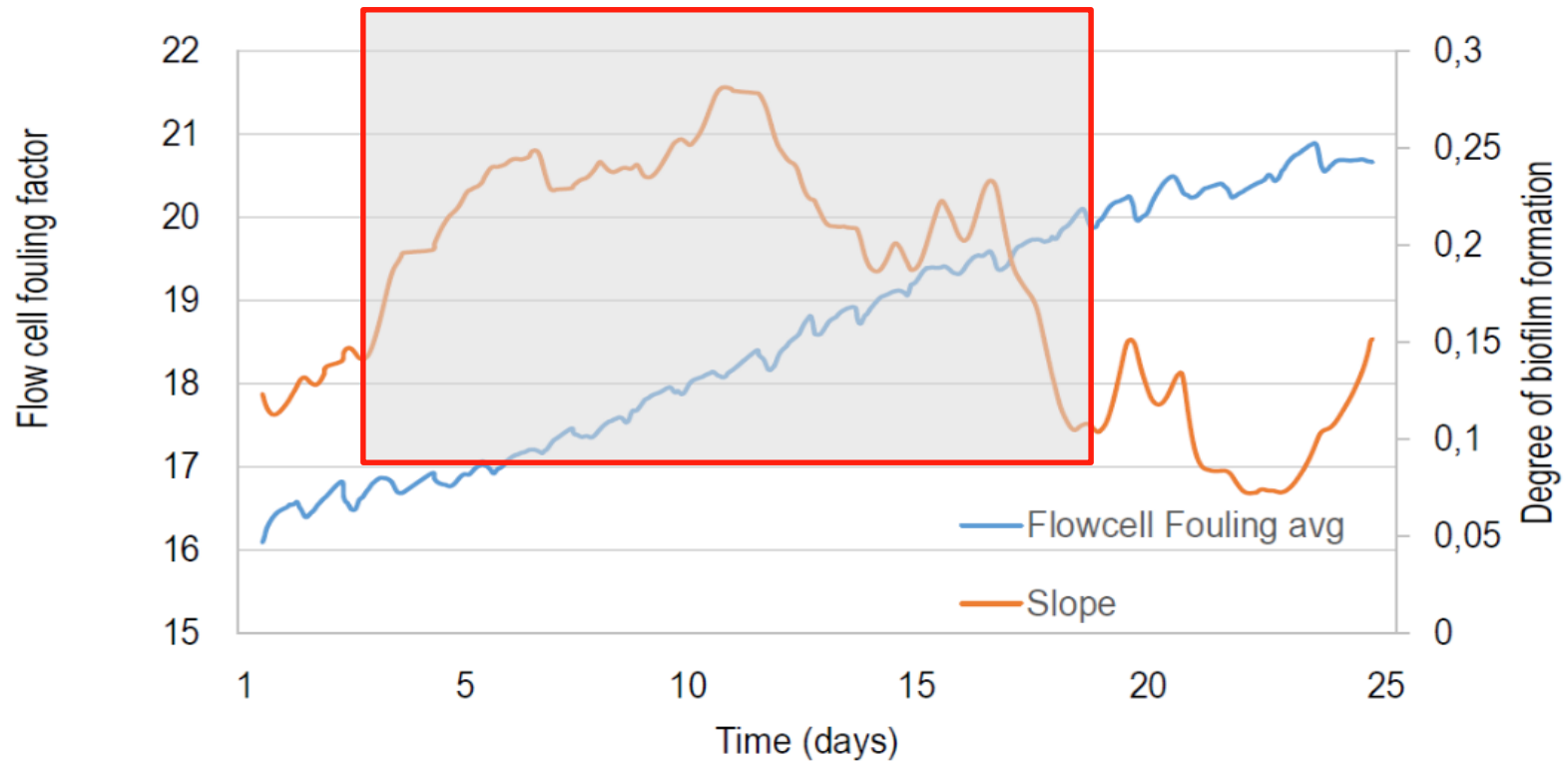


# Resultater - biofilm



# BACMON - biofilmudvikling

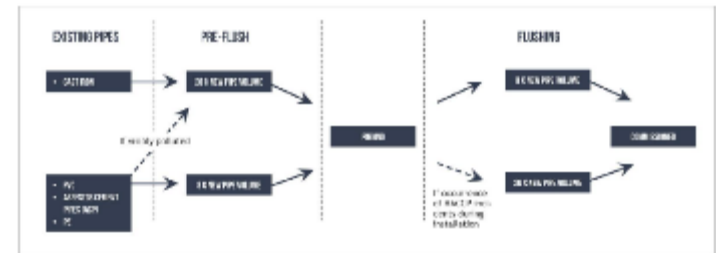
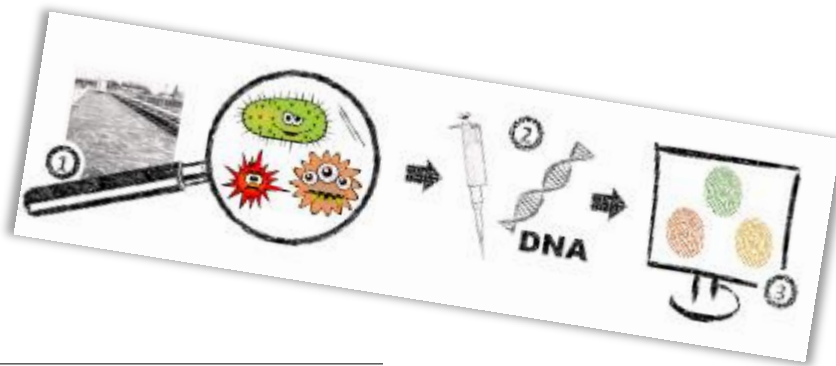
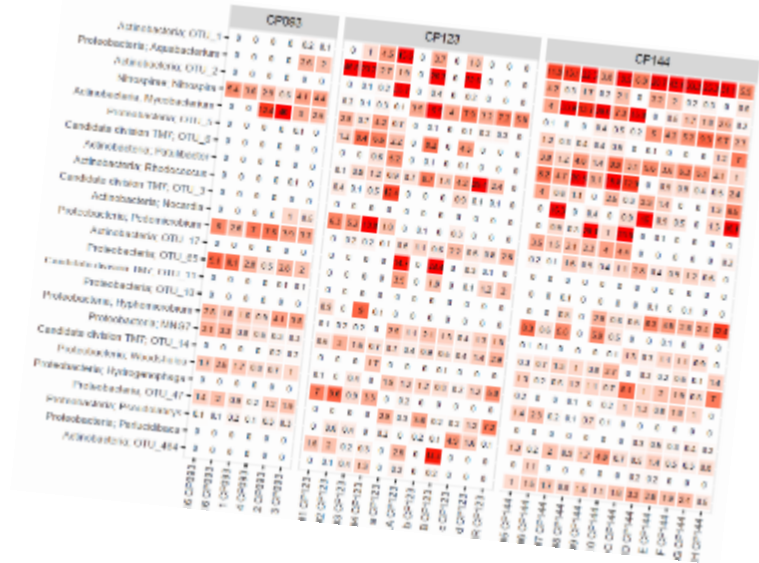
## mobilBACMON





# Det videnskabelige arbejde fortsætter...

- VTUF rapport udarbejdet
- Intern procedure for idriftsættelse udarbejdet hos Aarhus Vand
- 3 videnskabelige artikler er under udarbejdelse...



# Biofilm i drikkevandssystemer: Optimeret idriftsættelse af rør og tanke og videreudvikling af biofilmsensor

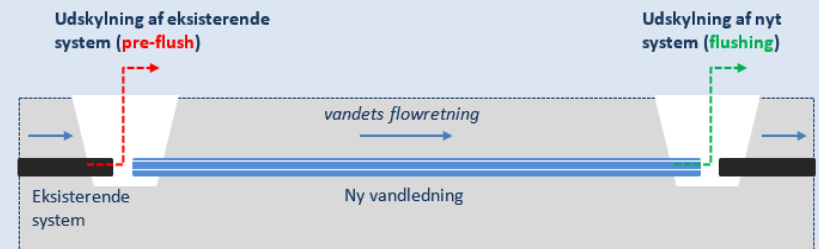
*Afrapportering for projekt støttet af  
VTU-Fonden*

31-10-2018

**Vandsektorens**  
Teknologiviklingsfond

# VTUF rapport

## Procedure for idriftsættelse af PE rør



### Pre-flush

**8 x volumen**

Der skylles med minimum 8 x volumen af den nye vandledding

(eller indtil vandet er synligt rent)

**svampning**

alle ledningsarbejder skal rengøres med svamp

### Flushing

**8 x volumen**

Der skylles med minimum 8 x volumen af den nye vandledding

(eller indtil vandet er synligt rent)

### Tæthedsprøvning

Alle nye ledningsanlæg tæthedsprøves

### Vandprøver

Der udtages som udgangspunkt kun vandprøver på transmissions- og råvandsledninger

# Svar på problemstillingerne

1. Forbruget af mandetimer, analyser, vandforbrug etc. er **blevet kortlagt og evalueret** i projektet
2. De observerede **"kimtals-toppe"** er et udbredt fænomen ved alle indsættelser af PE materiale i vandbanen
3. Resultaterne viser **ikke at "kimtals-toppene" er et problem** for vandkvaliteten
4. En **ny idriftsættelsesprocedure** for nye PE rør er blevet udarbejdet, testet og sat i værk
5. GRUNDFOS BACMON gav **hurtige og præcise svar** på hvornår skylningen af den nye ledning er tilstrækkelig



# Contact details



Bring ideas to life  
VIA University College

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Vand & Klima

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<https://en.via.dk/>



aarhusvand

