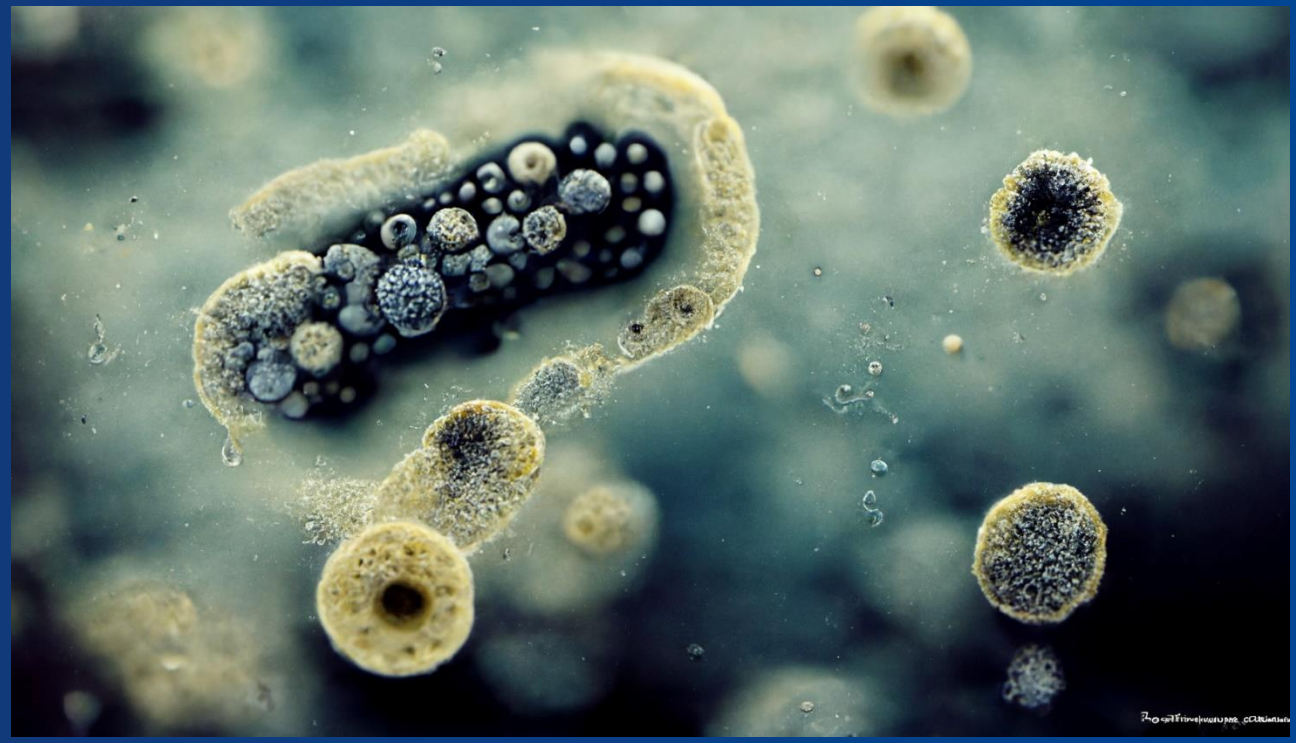
Emerging Methods for Assessing Bacterial Growth Potential



Cynthia Hallé & Michael Waak

16. October 2023

Biostabilitet i ledningsnett og tolkning av mikrobiologiske analyser

Norsk Vannforening - Olso

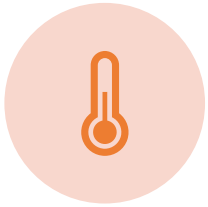
SINTEF NTNU

Biostability in distribution network

- What is biostability ?
  - The ability to limit regrowth in drinking water

- Factors influencing biostability

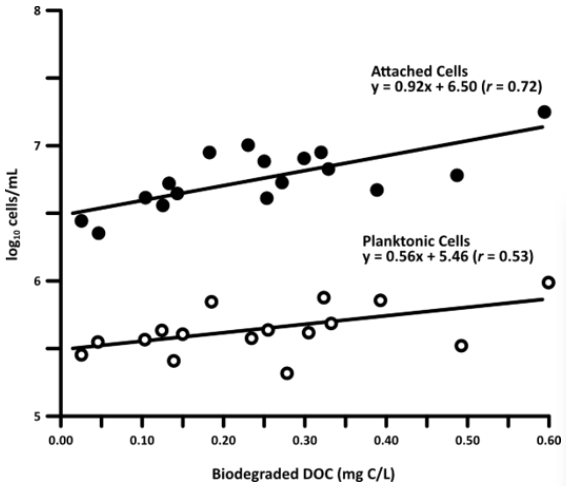
Water Age
(Residence Time)



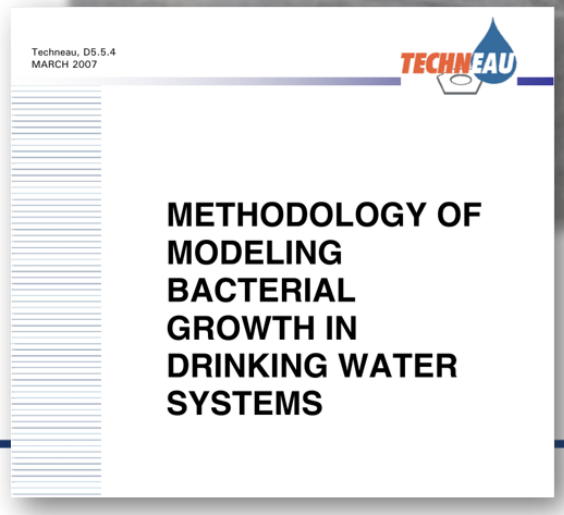
Temperature

Carbon & Nutrient
Availability

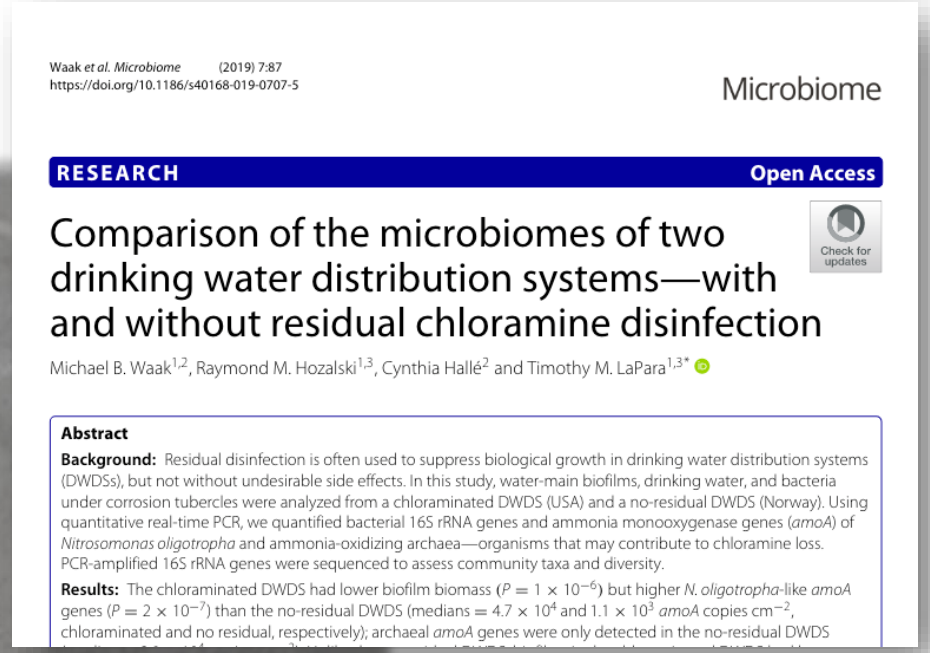
Bruaset S, Hem LJ. 2014. A206 Biostabilitet i drikkevannsnett. Norsk Vann; Report No.: 206/2014.



Mathieu et al. 2005. *Rev Des Sci De L'Eau*. 5:91–112. doi:10.7202/705155ar

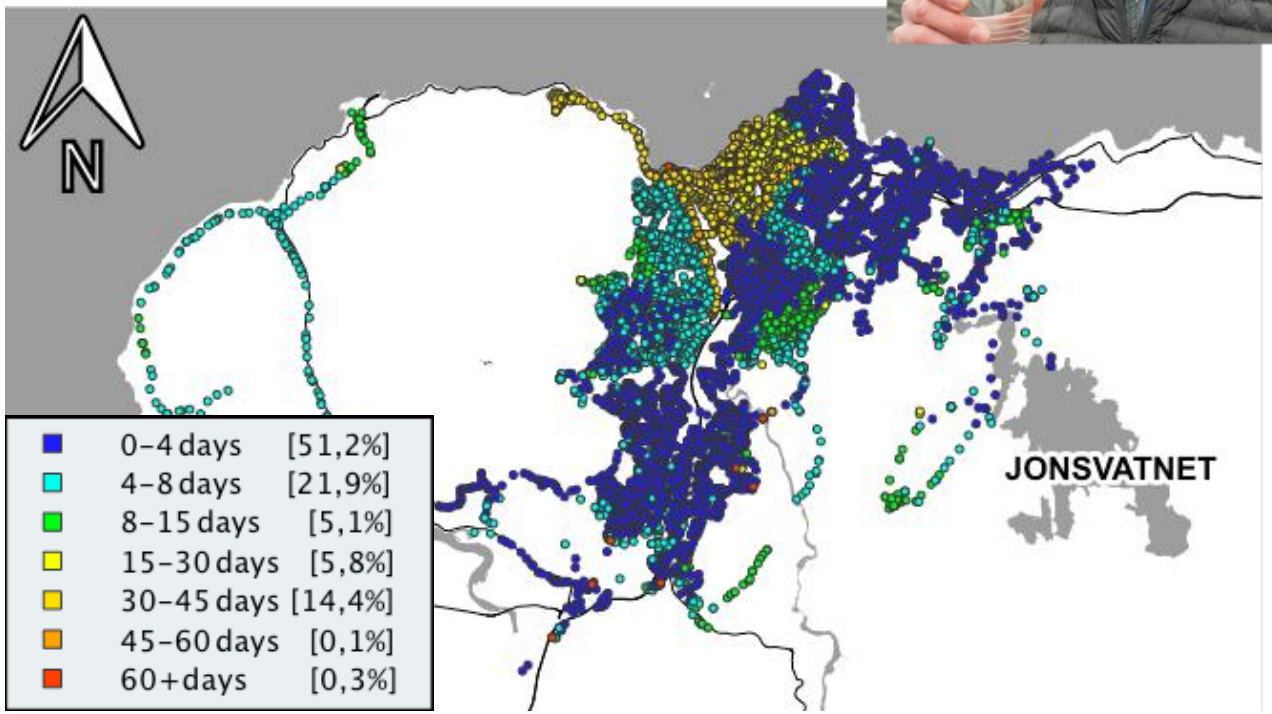


Rubulis et al.. 2007 Mar. Methodology of Modeling Bacterial Growth in Drinking Water Systems. (Techneau). Report No.: D 5.5.4.



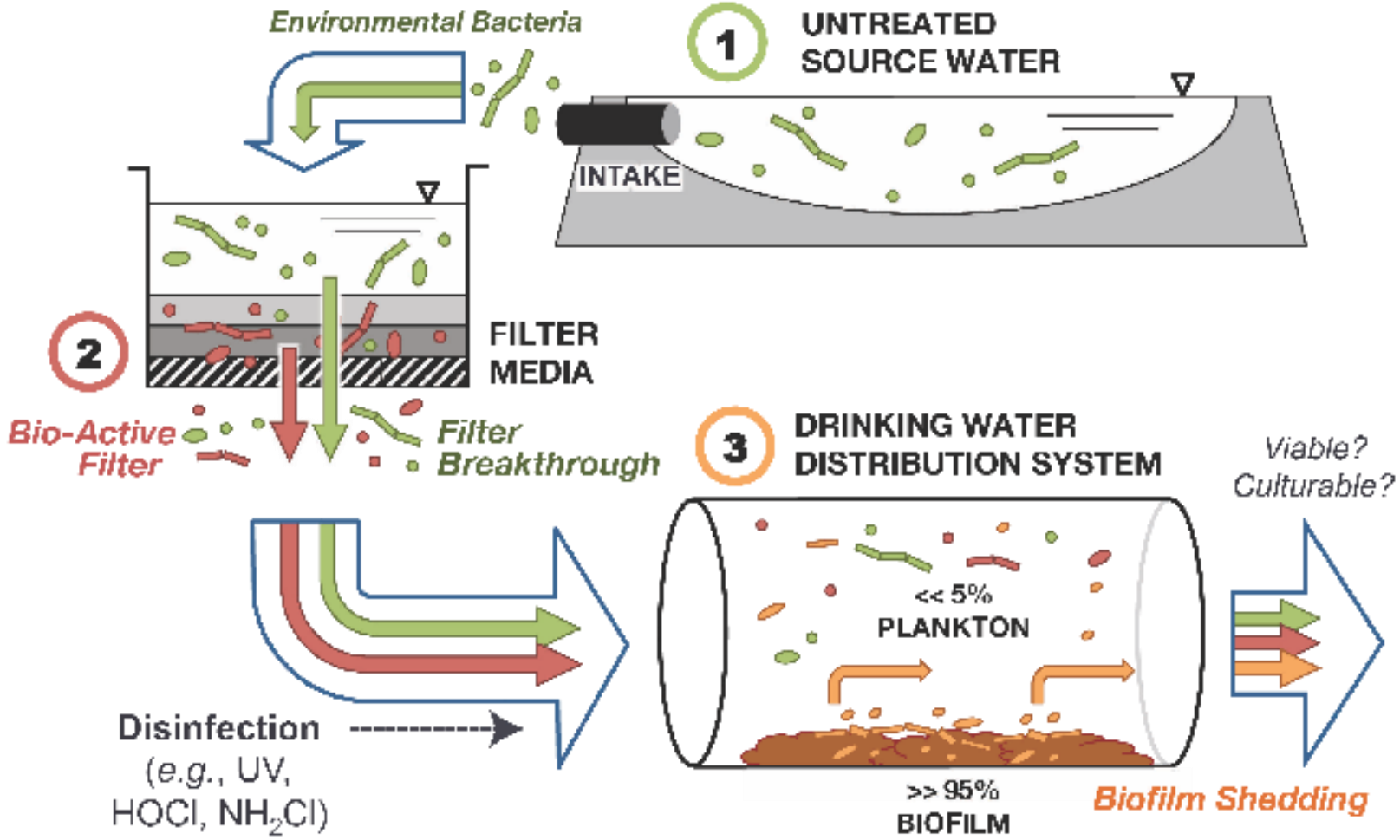
Water Age in Distribution Network

- Tracer study to determine water age – using salt from chlorine production
- Higher water age has higher HPC count



Rakstang JK, Waak MB, Rokstad MM, Hallé C. 2021. Demonstrating the potential of salt tracer studies to improve Norwegian drinking water network models and water age estimates. *Tidsskr VANN*. 56(1):61–69.

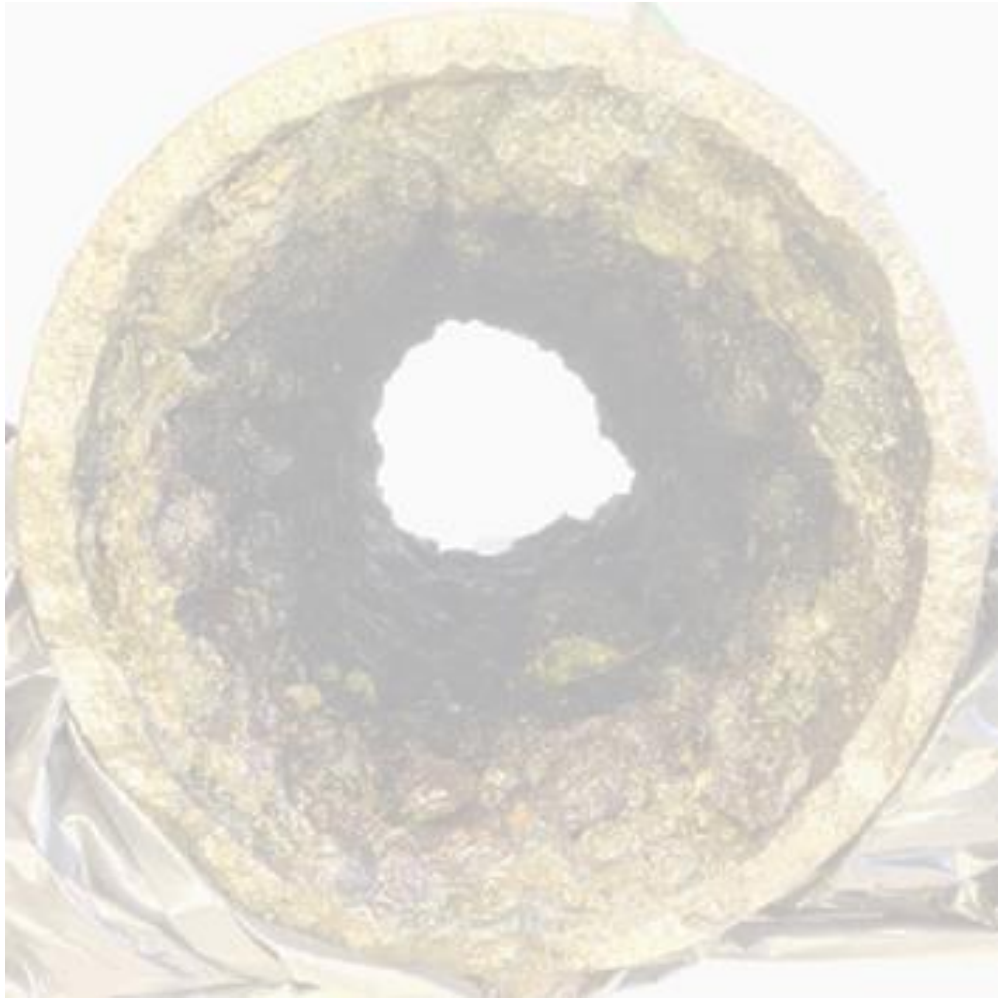
Sources of Biomass in the Network



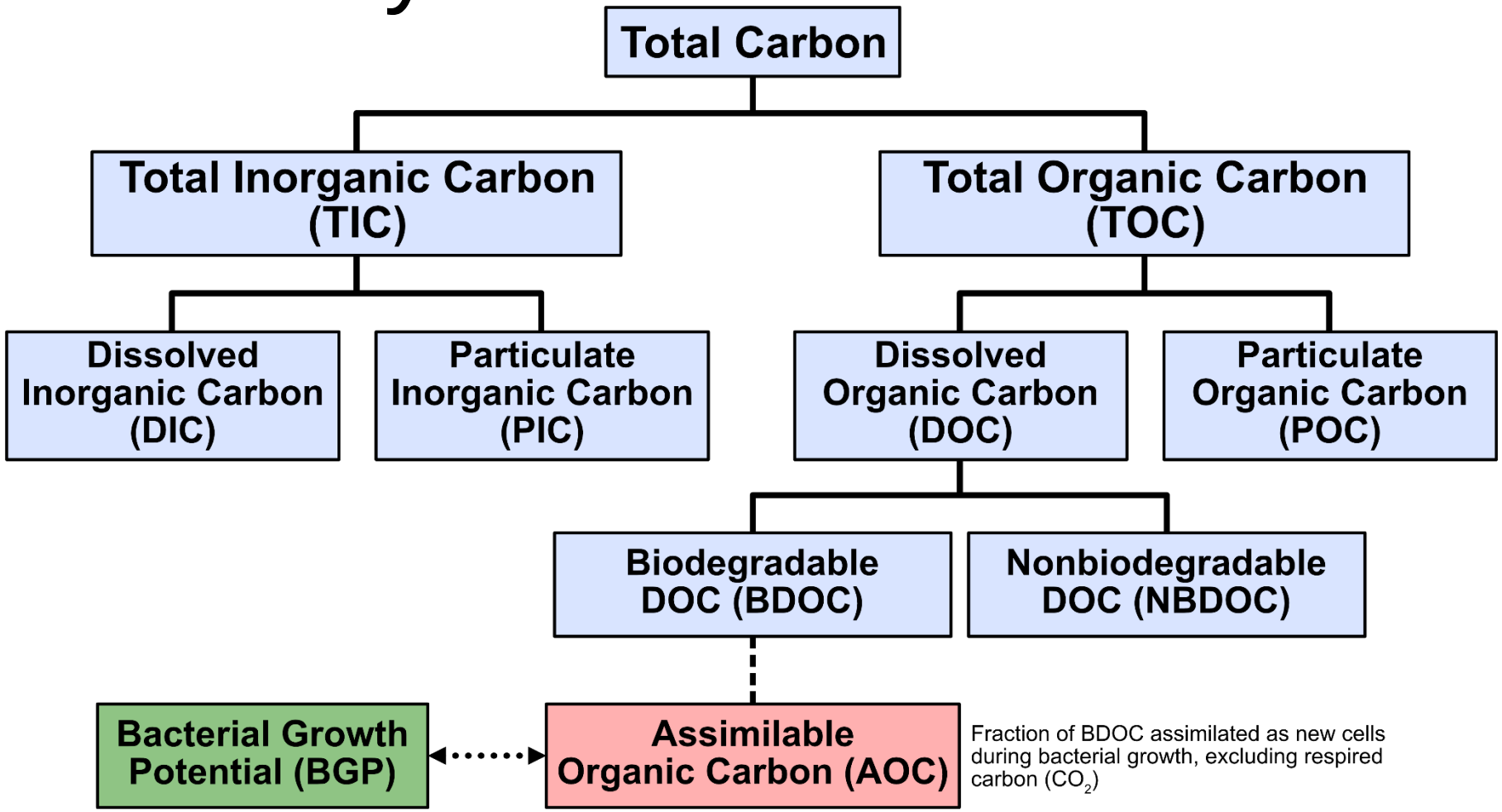
Assessing biostability

First methods focus on biodegradable organic carbon

- Assimilable organic carbon (AOC)
- Optimization of bioassay
- Use of indigenous bacterial community

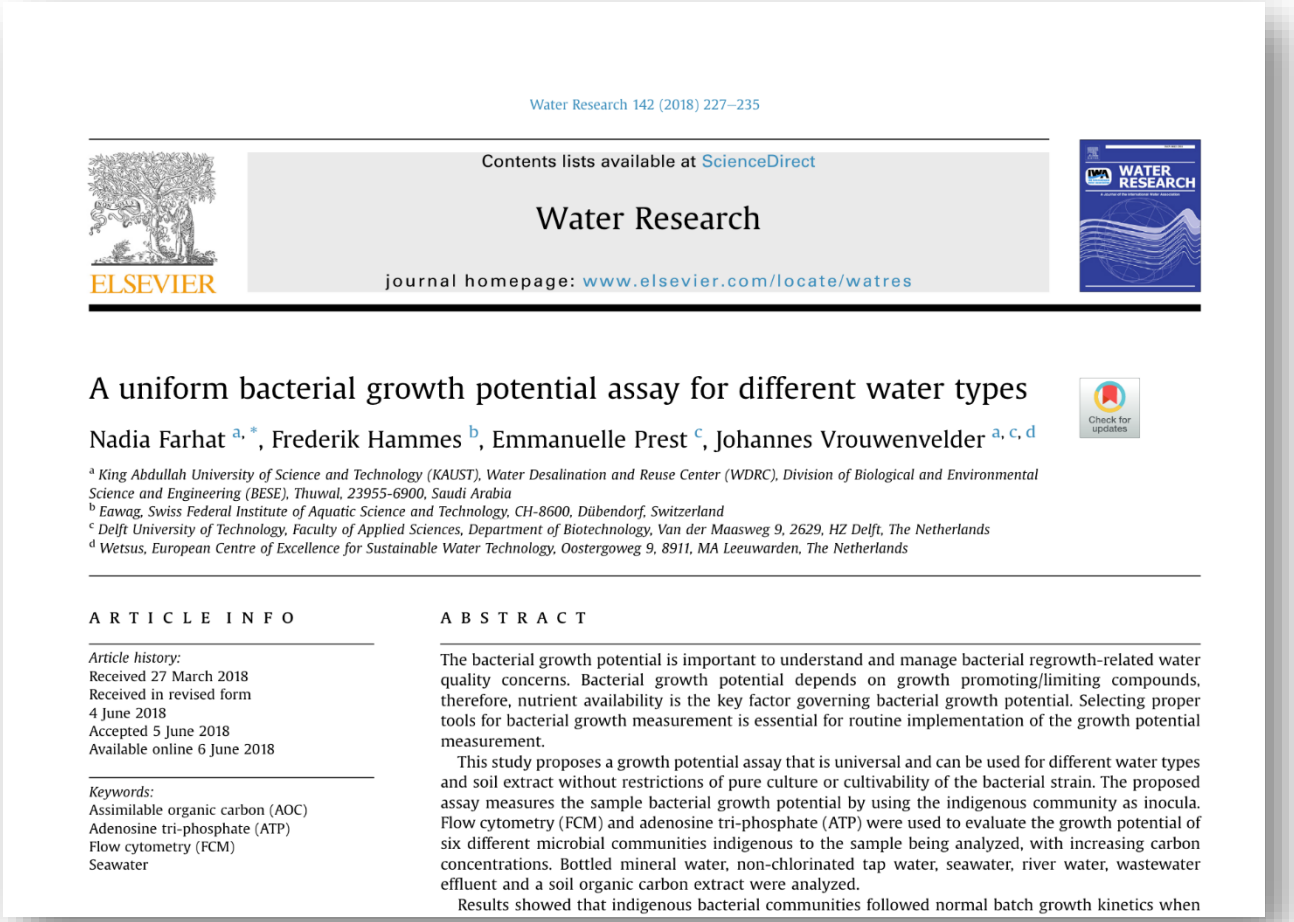


Carbon hierarchy



An alternative to AOC and BDOC

- Useful diagnostic parameter
- Versatile method
- Streamlined and simplified compared to AOC



Water Research 142 (2018) 227–235

Contents lists available at ScienceDirect

Water Research

journal homepage: www.elsevier.com/locate/watres

A uniform bacterial growth potential assay for different water types

Nadia Farhat [a, *], Frederik Hammes [b], Emmanuelle Prest [c], Johannes Vrouwenvelder [a, c, d]

[a] King Abdullah University of Science and Technology (KAUST), Water Desalination and Reuse Center (WDRC), Division of Biological and Environmental Science and Engineering (BESE), Thuwal, 23955-6900, Saudi Arabia
[b] Eawag, Swiss Federal Institute of Aquatic Science and Technology, CH-8600, Dübendorf, Switzerland
[c] Delft University of Technology, Faculty of Applied Sciences, Department of Biotechnology, Van der Maasweg 9, 2629, HZ Delft, The Netherlands
[d] Wetsus, European Centre of Excellence for Sustainable Water Technology, Oostergoweg 9, 8911, MA Leeuwarden, The Netherlands

ARTICLE INFO

Article history:
Received 27 March 2018
Received in revised form
4 June 2018
Accepted 5 June 2018
Available online 6 June 2018

Keywords:
Assimilable organic carbon (AOC)
Adenosine tri-phosphate (ATP)
Flow cytometry (FCM)
Seawater

ABSTRACT

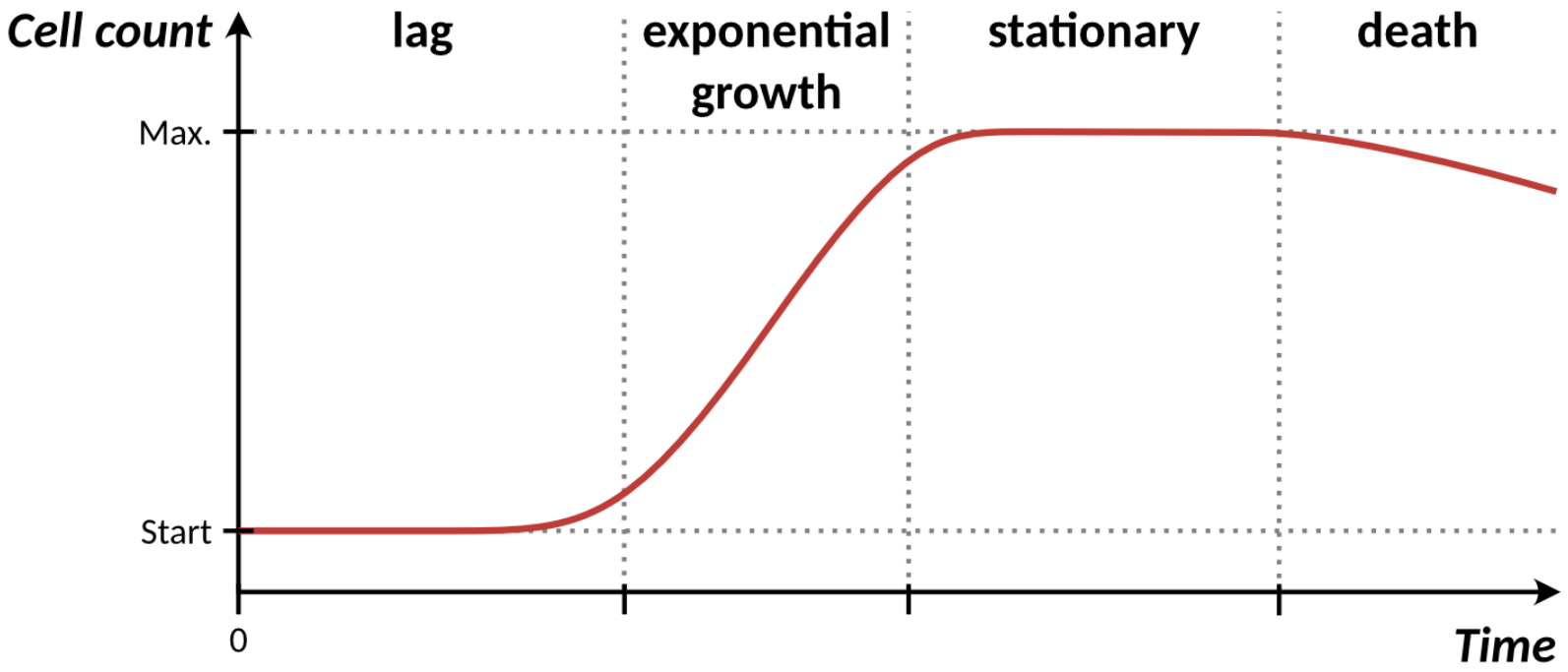
The bacterial growth potential is important to understand and manage bacterial regrowth-related water quality concerns. Bacterial growth potential depends on growth promoting/limiting compounds, therefore, nutrient availability is the key factor governing bacterial growth potential. Selecting proper tools for bacterial growth measurement is essential for routine implementation of the growth potential measurement.

This study proposes a growth potential assay that is universal and can be used for different water types and soil extract without restrictions of pure culture or cultivability of the bacterial strain. The proposed assay measures the sample bacterial growth potential by using the indigenous community as inocula. Flow cytometry (FCM) and adenosine tri-phosphate (ATP) were used to evaluate the growth potential of six different microbial communities indigenous to the sample being analyzed, with increasing carbon concentrations. Bottled mineral water, non-chlorinated tap water, seawater, river water, wastewater effluent and a soil organic carbon extract were analyzed.

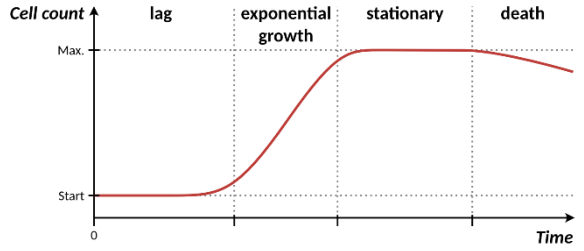
Results showed that indigenous bacterial communities followed normal batch growth kinetics when

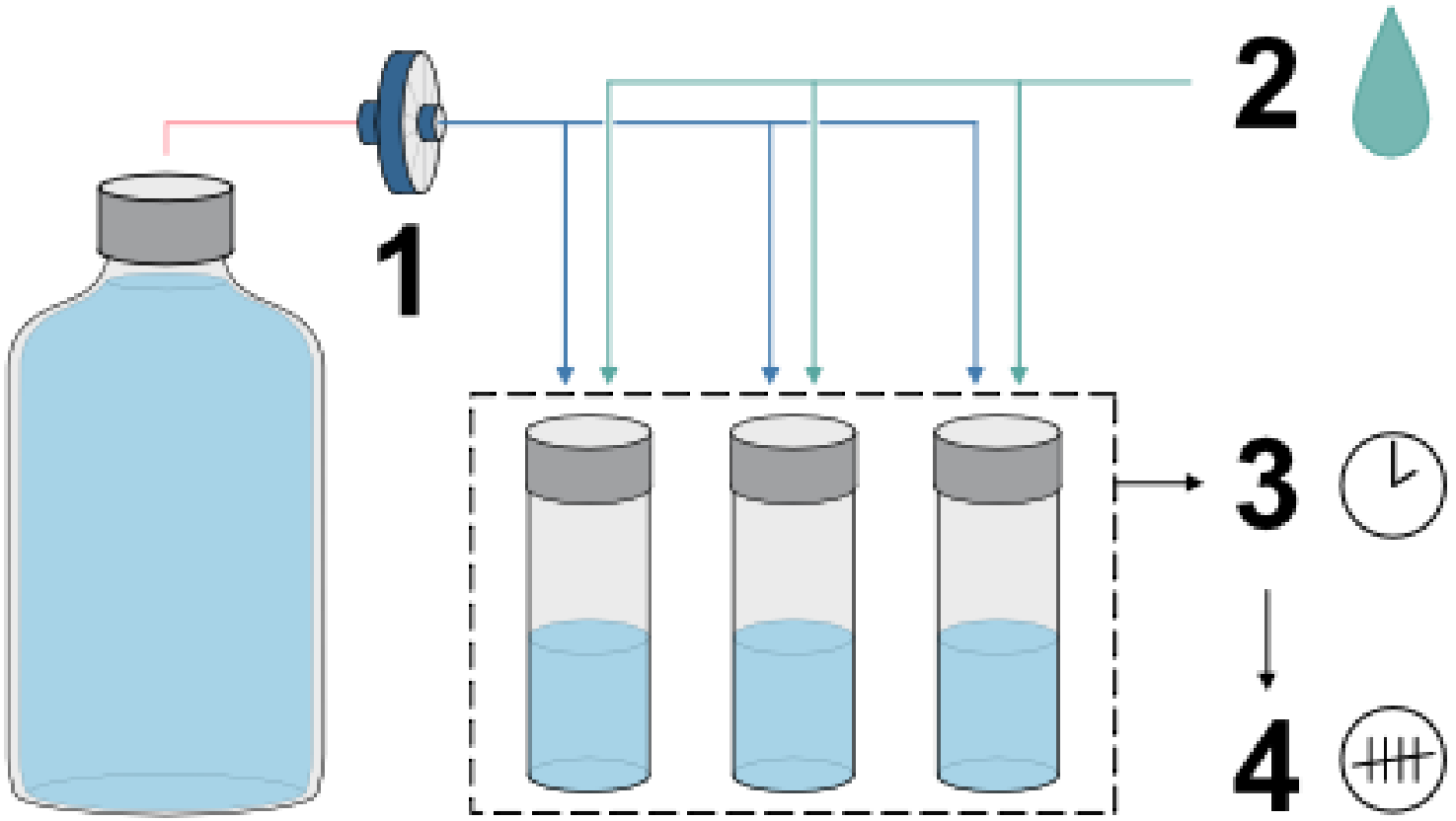
Farhat et al. (2018) doi: 10.1016/j.watres.2018.06.010

Bacterial Growth Curve



Bacterial Growth Potential





1. Sterilization
2. Inoculation
3. Incubation
4. Enumeration

Enumeration - Flow Cytometry

- Automated flow cytometry using cartridge system
  - All reagents and waste products are contained
  - Ca. 1000 reads per cartridge
- Continuous (online) and manual modes
- Portable, with built-in computer, touch screen

BactoSense

bNovate TECHNOLOGIES

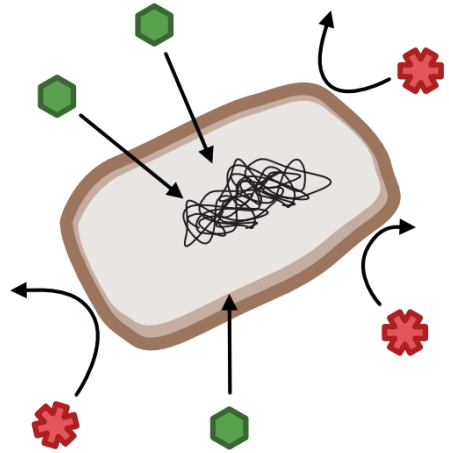
SINTEF NTNU

Incubation with intercalating dyes

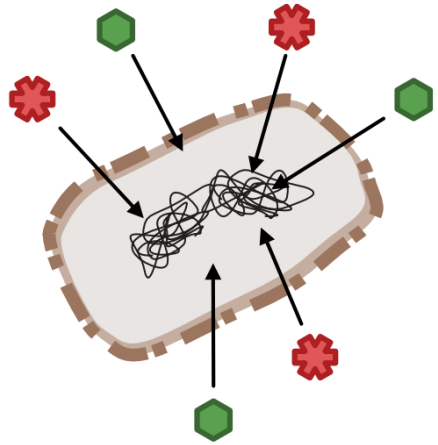
SYBR Green I
(membrane permeable)

Propidium iodide
(membrane impermeable)

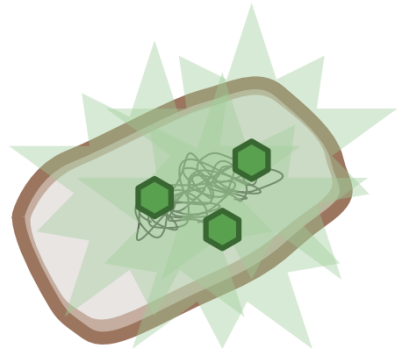
Intact cell membrane

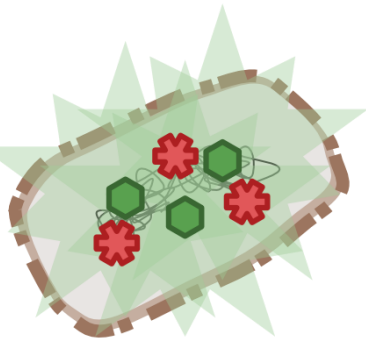


Damaged cell membrane

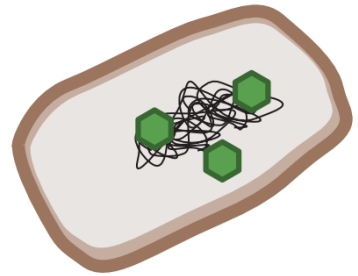


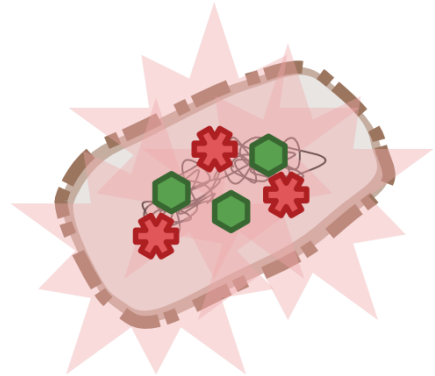
SYBR Green I
emits **green light**
(FL1 / 525 nm)

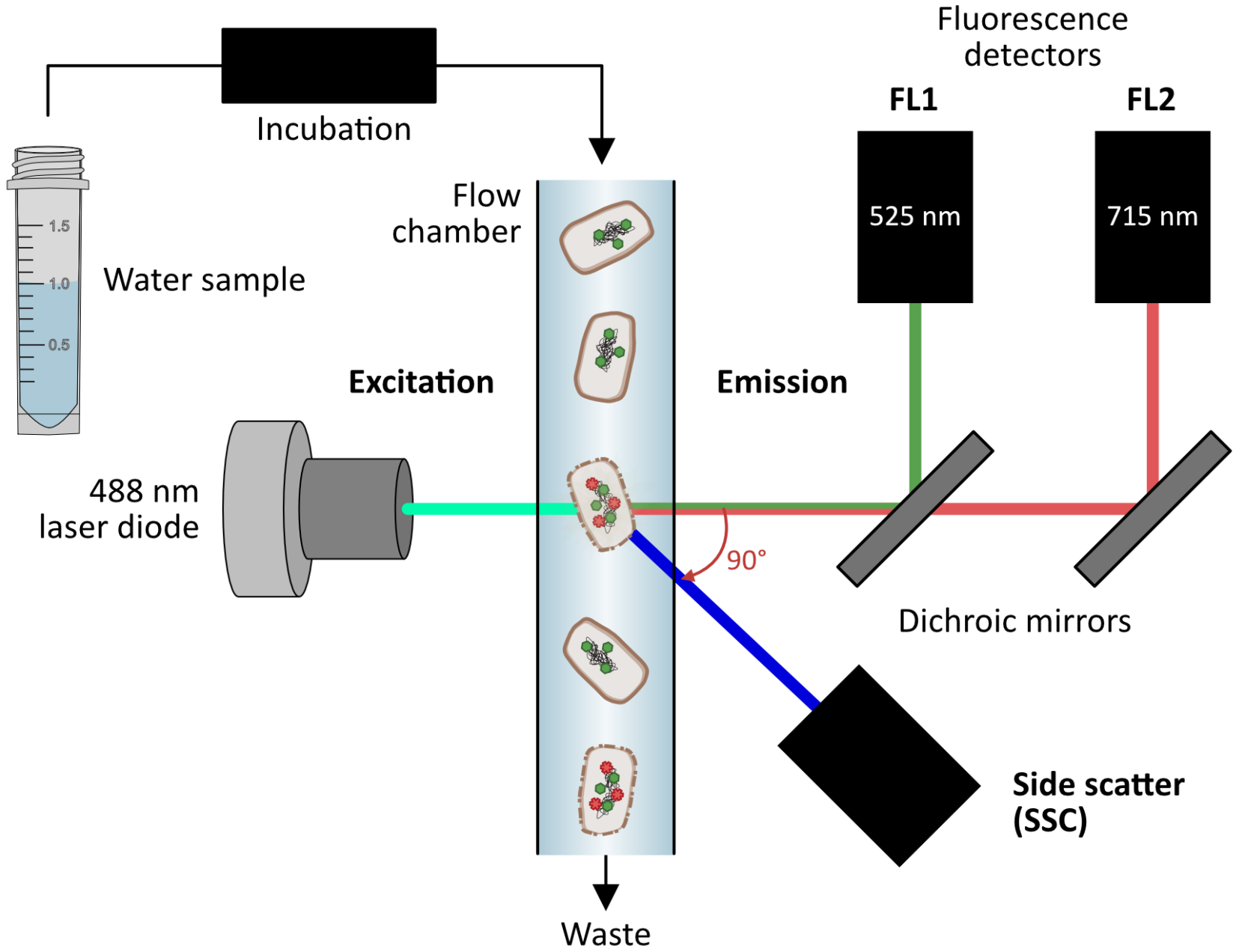




Propidium iodide
emits **red light**
(FL2 / 715 nm)

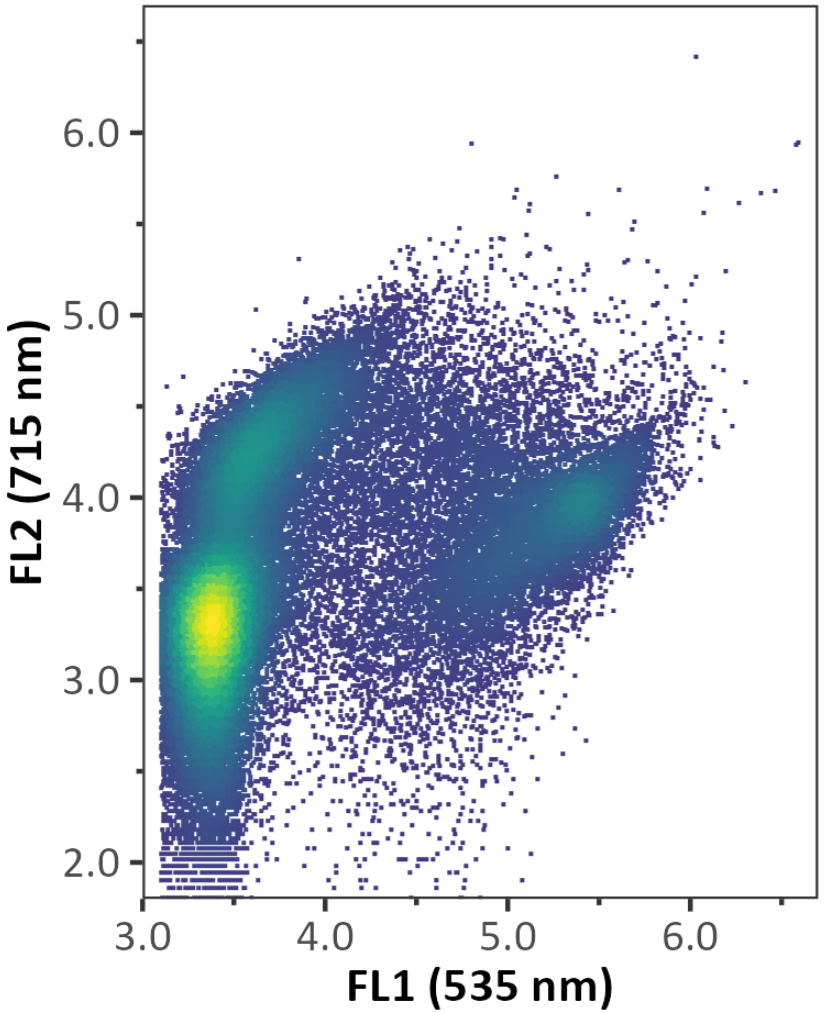




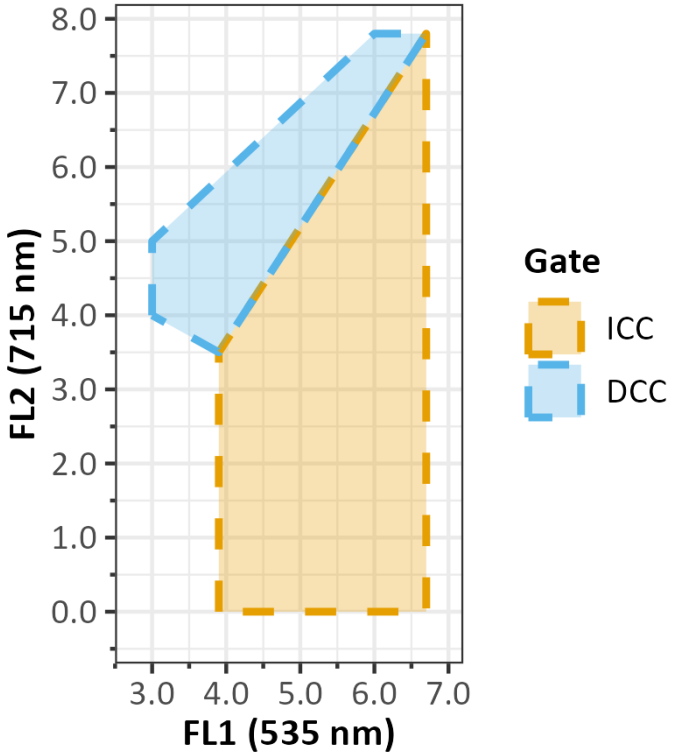

Fluorescence detectors
FL1
FL2
Incubation
Flow chamber
525 nm
715 nm
Water sample
Excitation
Emission
488 nm laser diode
90°
Dichroic mirrors
Side scatter (SSC)
Waste

Damaged cells
propidium iodide,
red fluorescence

“Dot plot” or “cytogram”



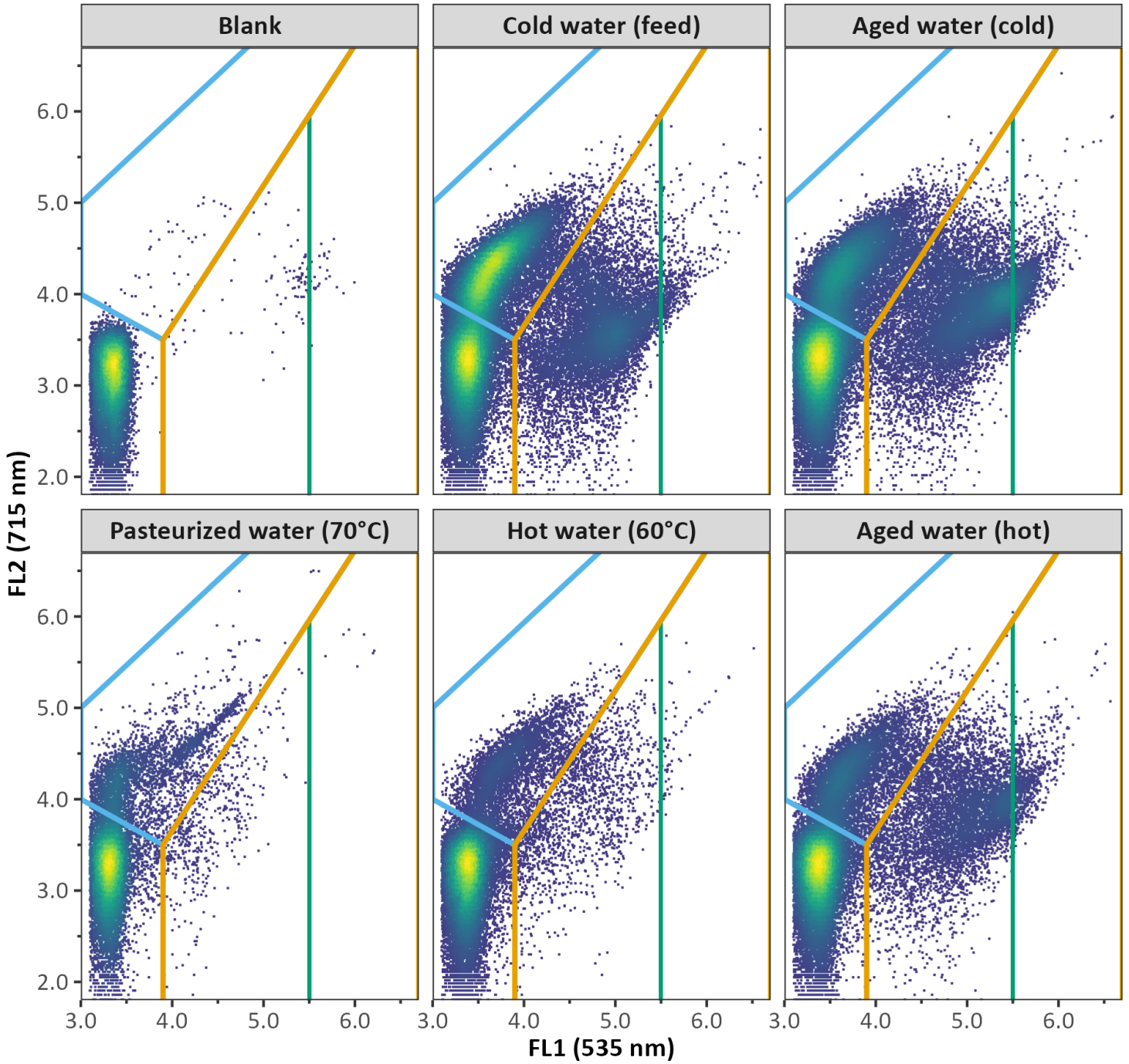
All cells
SYBR Green I,
green fluorescence

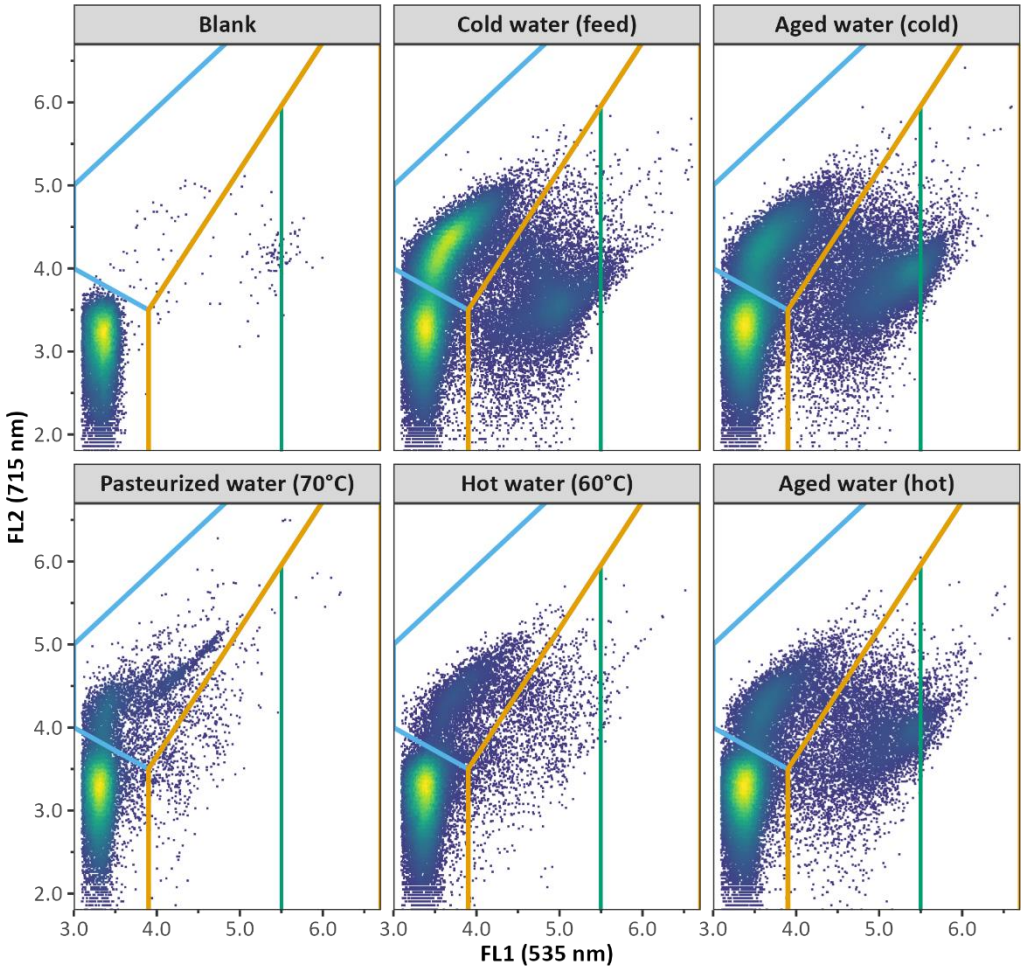


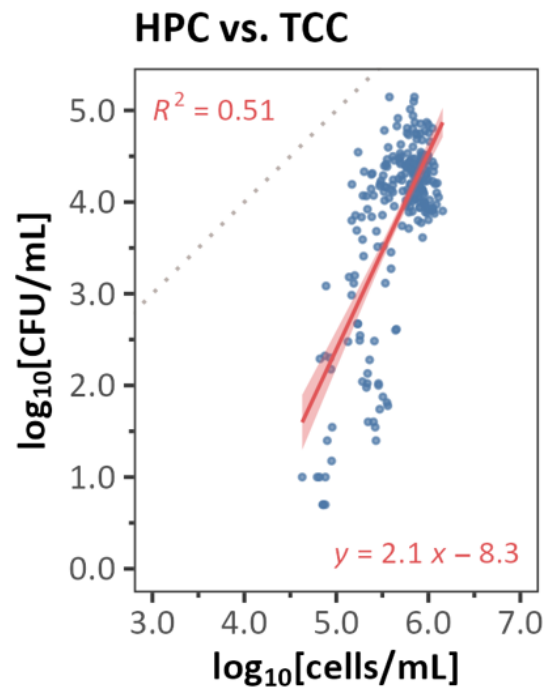
DCC damaged cell count
ICC intact cell count

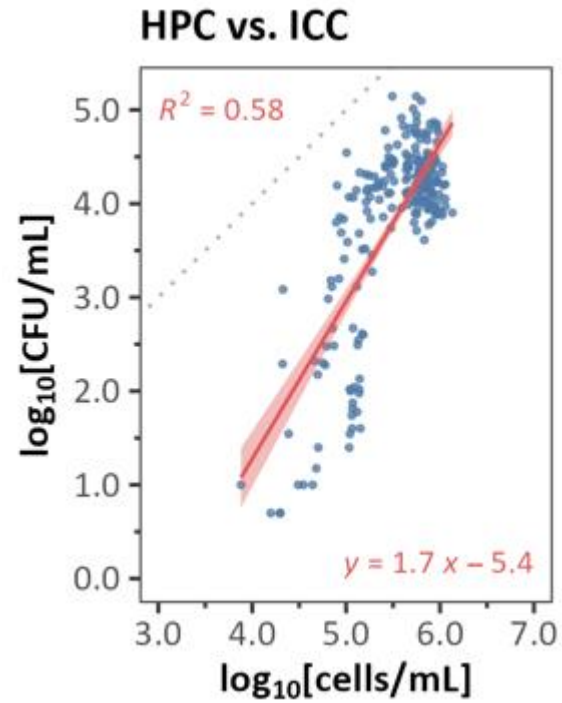
TCC total cell count

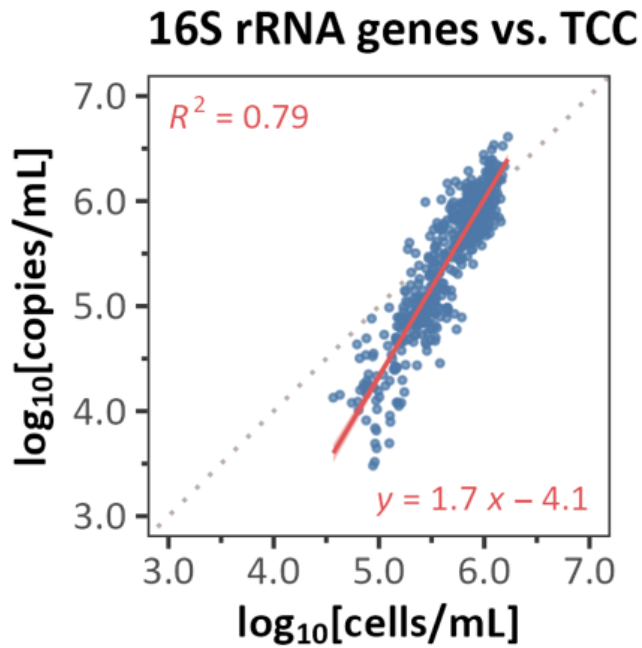
TCC = ICC + DCC

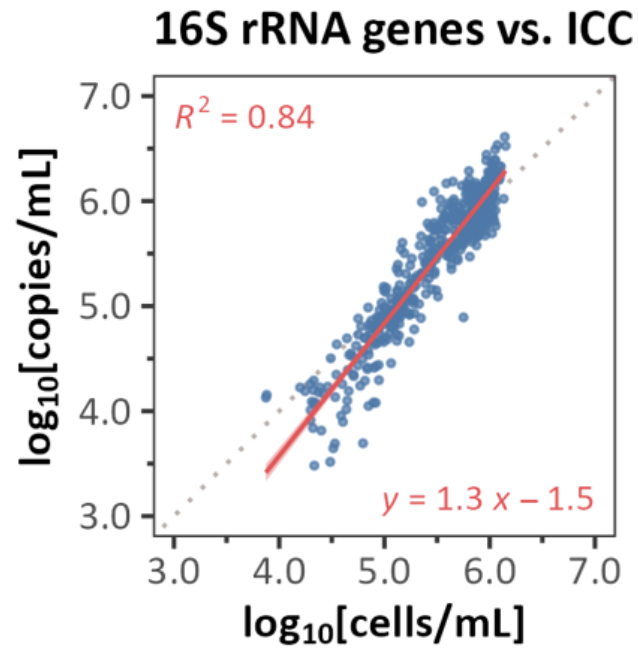

Blank
Cold water (feed)
Aged water (cold)
Pasteurized water (70°C)
Hot water (60°C)
Aged water (hot)
FL2 (715 nm)
FL1 (535 nm)
2.0
3.0
4.0
5.0
6.0


Blank
Cold water (feed)
Aged water (cold)
Pasteurized water (70°C)
Hot water (60°C)
Aged water (hot)
FL2 (715 nm)
FL1 (535 nm)

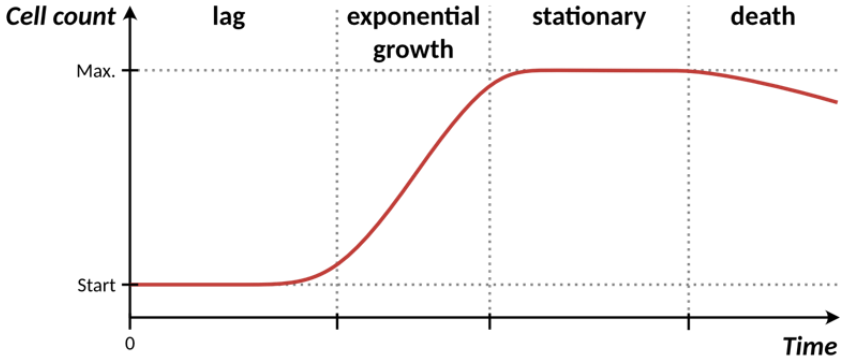

HPC vs. TCC
R^2 = 0.51
y = 2.1 x – 8.3
log10[CFU/mL]
log10[cells/mL]

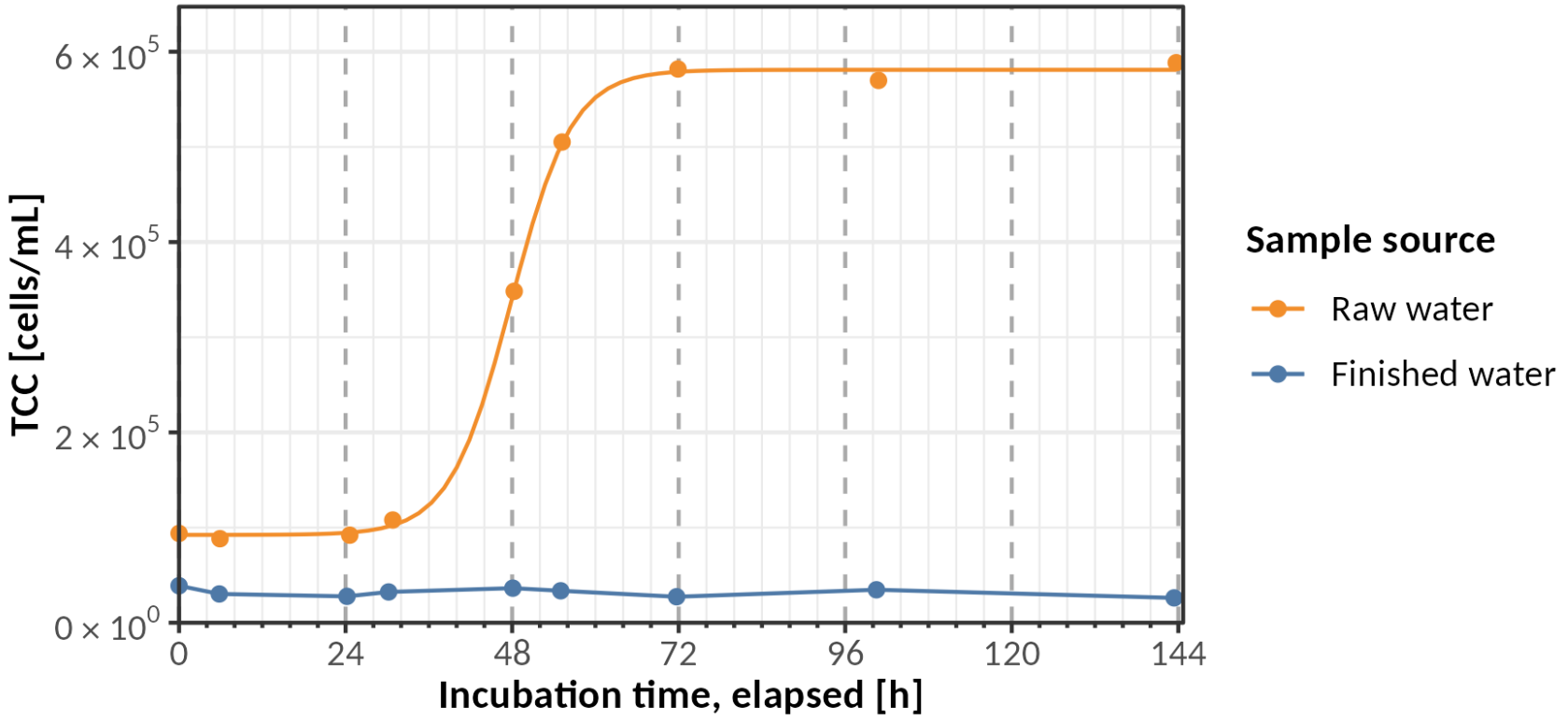

HPC vs. ICC
R^2 = 0.58
y = 1.7 x – 5.4
log10[CFU/mL]
log10[cells/mL]

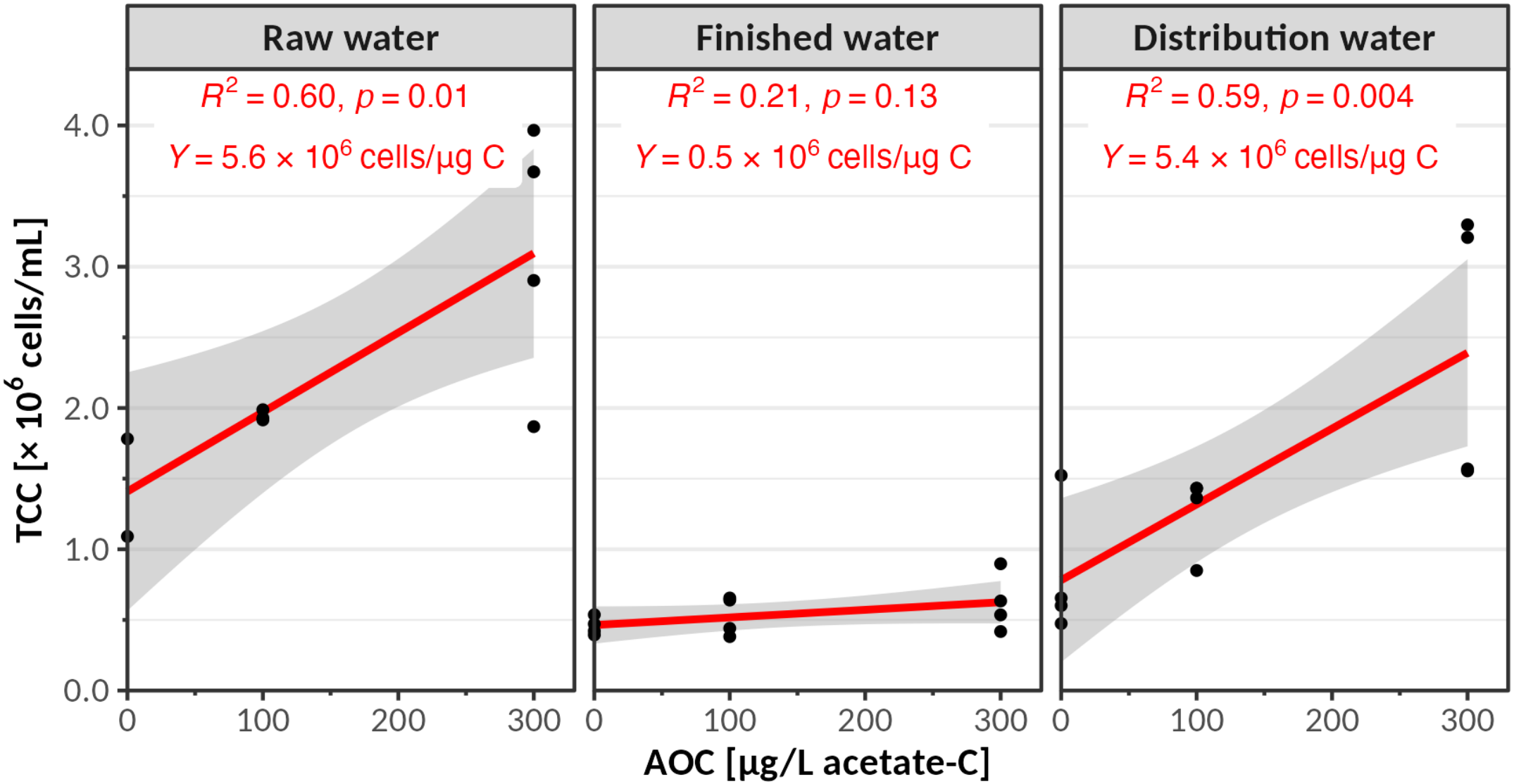

16S rRNA genes vs. TCC
R^2 = 0.79
y = 1.7 x – 4.1
log10[copies/mL]
log10[cells/mL]


16S rRNA genes vs. ICC
R^2 = 0.84
y = 1.3 x – 1.5
log10[copies/mL]
log10[cells/mL]

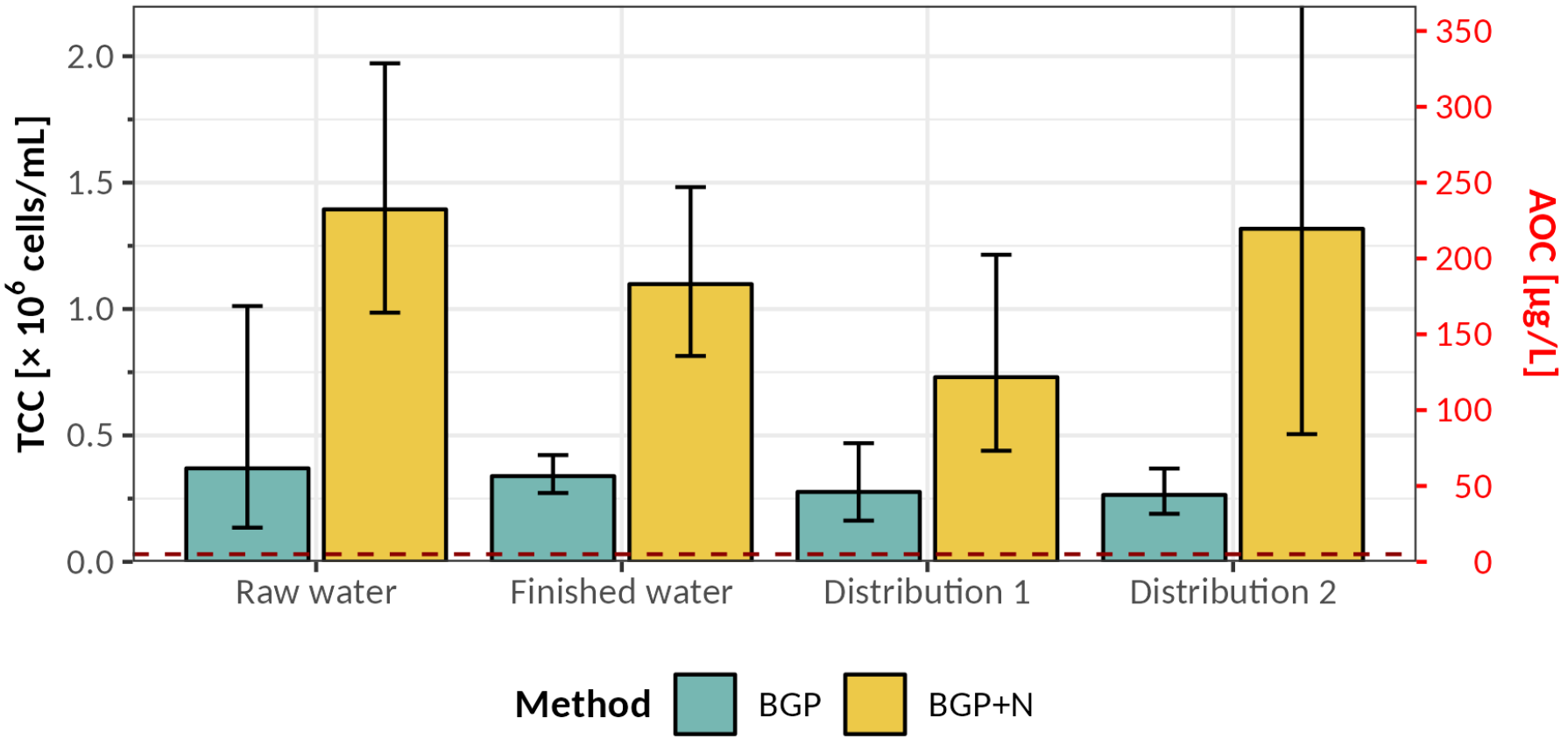
Results
Bacterial Growth Potential






Raw water
$R^2 = 0.60$, $p = 0.01$
$Y = 5.6 \times 10^6$ cells/µg C
Finished water
$R^2 = 0.21$, $p = 0.13$
$Y = 0.5 \times 10^6$ cells/µg C
Distribution water
$R^2 = 0.59$, $p = 0.004$
$Y = 5.4 \times 10^6$ cells/µg C
TCC [× 10^6 cells/mL]
0.0
1.0
2.0
3.0
4.0
0
100
200
300
AOC [µg/L acetate-C]

Nutrients vs. Carbon: Which limits bacterial growth?



Y_{AOC} = 6 × 10^6 cells/(µg C)

Conclusions

- Indigenous community bacterial growth potential assay
  - Simple and flexible
  - Consistent cell count enumeration using flow cytometry
  - Reproducible results
  - Relationship with known AOC analysis

- Novel diagnostic tool
  - Role to nutrient
  - Rate of growth in the network

Acknowledgment

Théophile Gourlin
Nina Barougier-Picolet
Alison Brice
Oslo VAV

Contact information

Cynthia Hallé cynthia.halle@ntnu.no

Michael Waak michael.waak@sintef.no