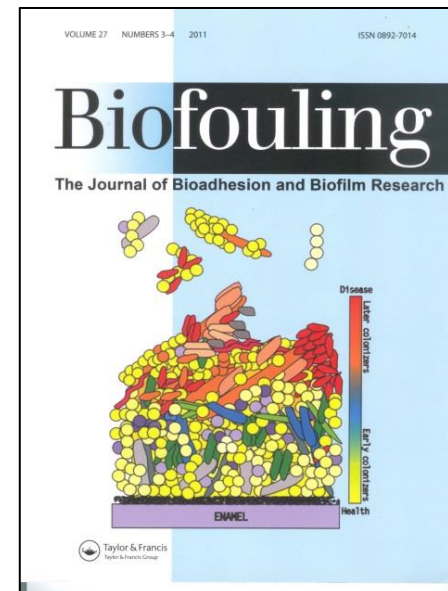


# Biofouling and Antifouling Measures



# What is fouling and biofouling?

## ➤ **Fouling: undesirable deposition of abiotic material on surfaces**

- Inorganic fouling („scaling“; precipitation of inorganic substances),
- Organic fouling (deposition of fat, oil, proteins etc.)
- Particle fouling (deposition of silt, clay, etc.)

## ➤ **Biofouling: undesirable accumulation of a biotic deposit on a surface**

(definition adopted from heat exchanger technology)

- Deposition and growth of **microorganisms** („microfouling“, microbial fouling).
- Deposition of **macroscopic organisms** („macrofouling“)

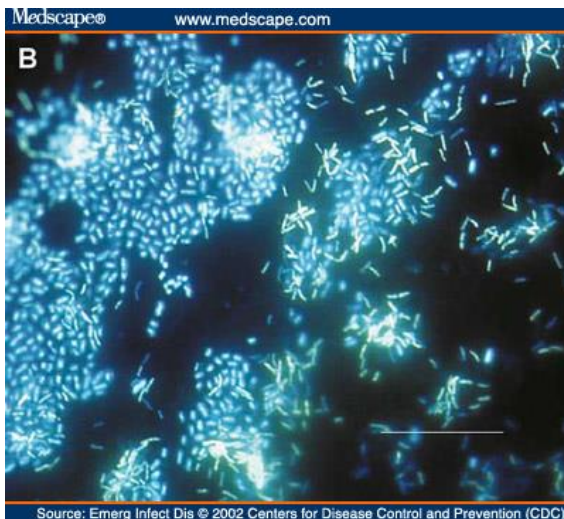
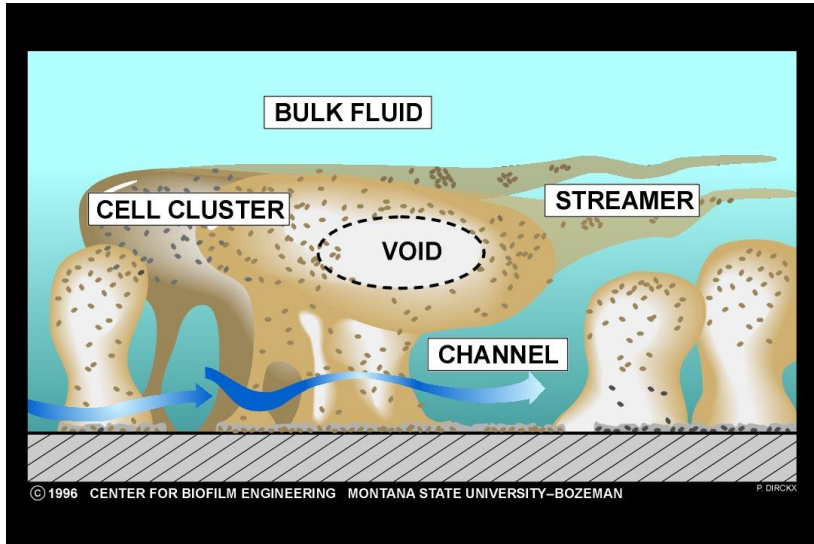
Operationally defined on the basis of process efficiency disturbances (loss of process performance, of product quality and quantity in combination with economic losses), in technical environments and in medical context.

The natural phenomenon underlying biofouling is **biofilm formation**.

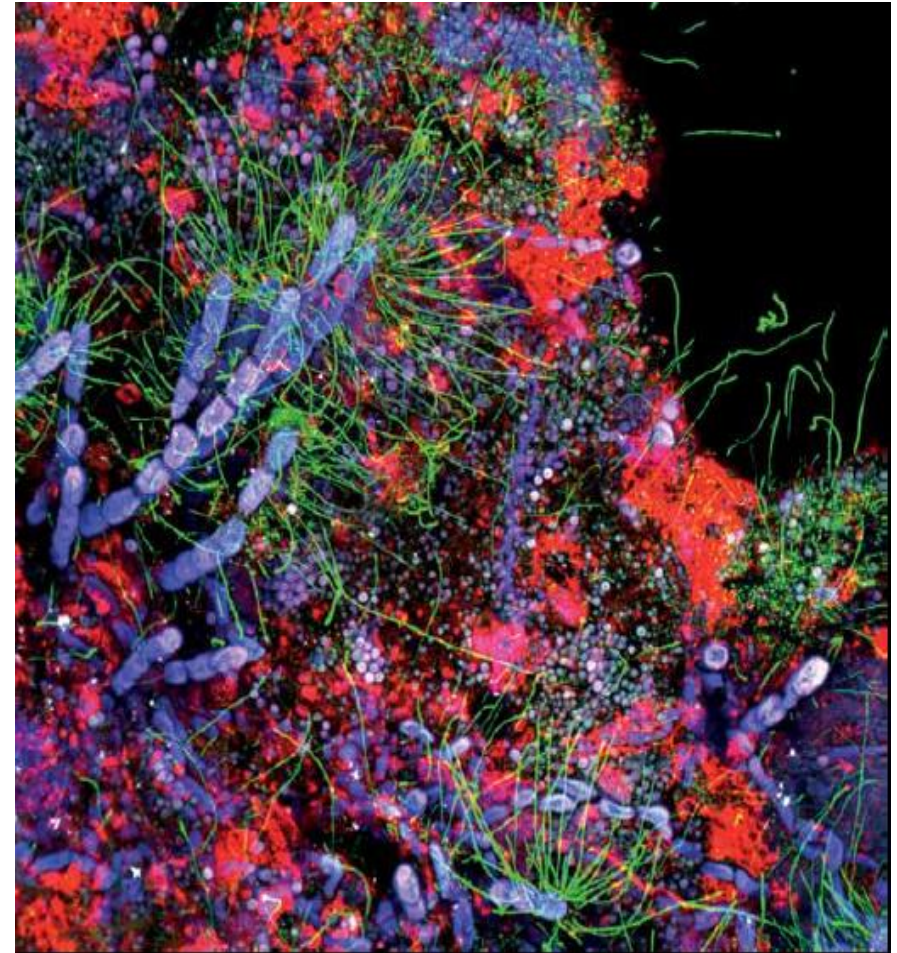


# Biofilms

Microbial communities living at interfaces, enclosed in a hydrated matrix of extracellular polymeric substances (EPS).



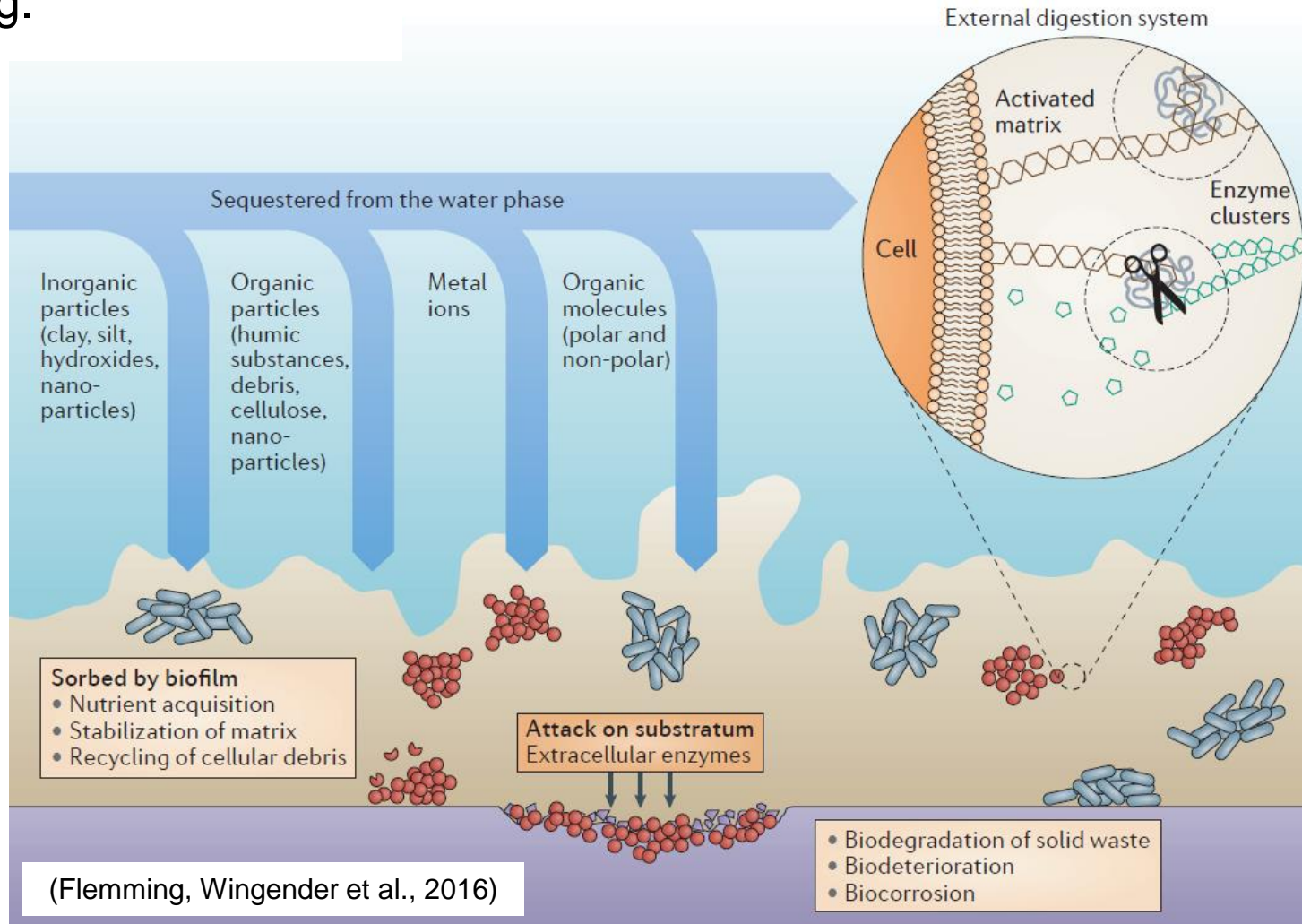
**Drinking water biofilm**



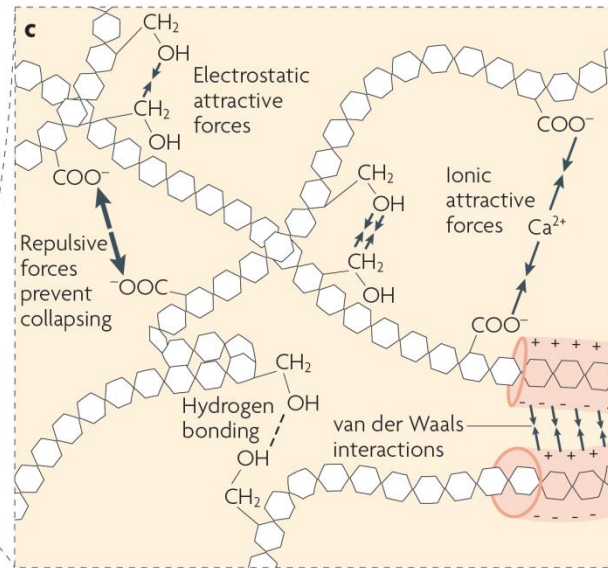
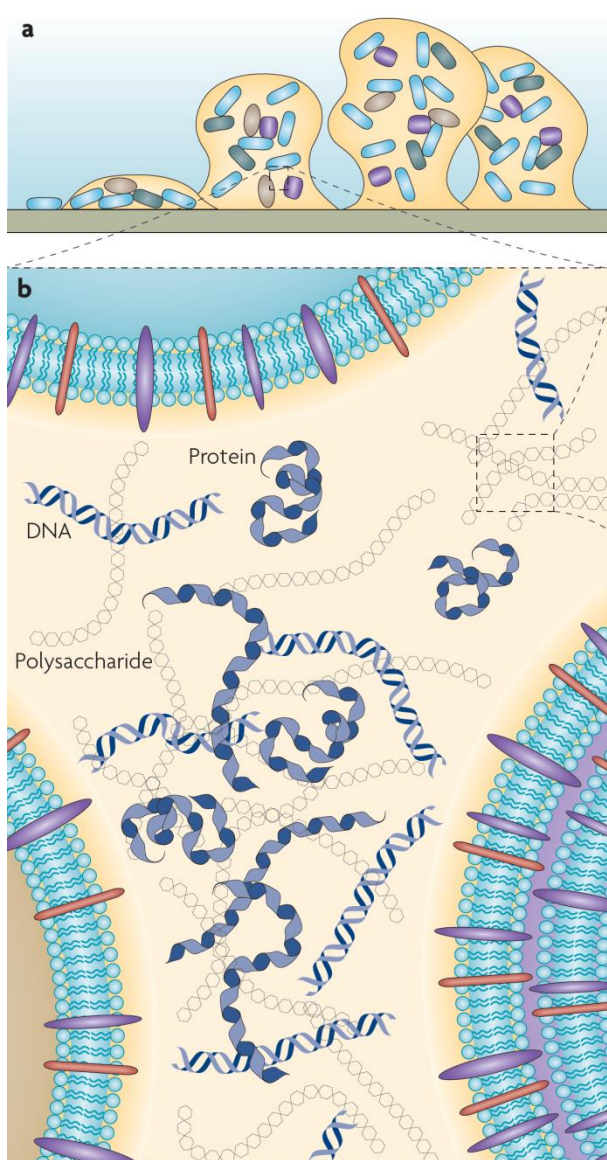
**River biofilm.** Nucleic acid stain: green; lectin stain: red (EPS matrix); autofluorescence of algae: blue; autofluorescence of cyanobacteria: purple/white. Image dimensions: 246  $\mu\text{m}$   $\times$  246  $\mu\text{m}$ . (Source: Kamjunke et al., 2015)

# Biofouling

**Biofouling:** Biofilm formation in the wrong place at the wrong time, often combined with capture and retention of inorganic and organic compounds/particles  $\Rightarrow$  coincidence of biofouling with non-biological fouling.



# EPS – key molecules of biofilm structure and function



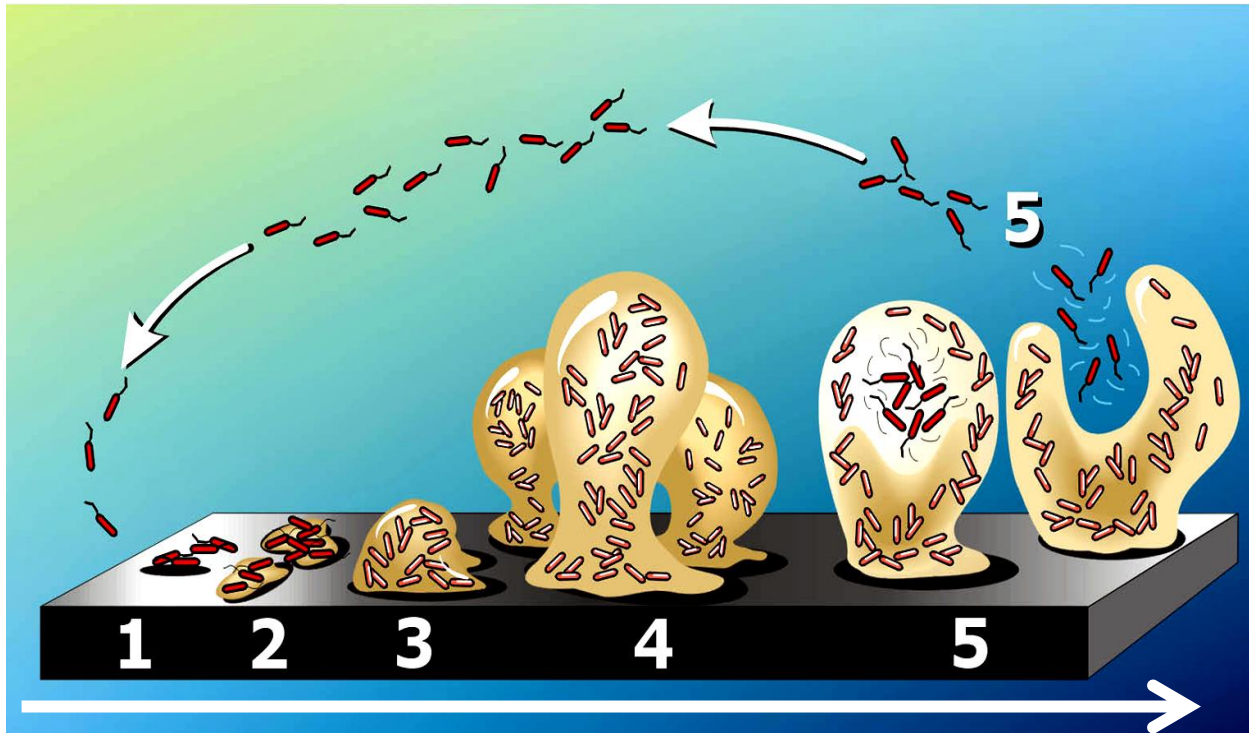
## Interactions between EPS

- Formation of hydrogel
- Responsible for mechanical stability of biofilms
- Responsible for viscoelastic properties of biofilms
- Important targets for antifouling measures.

- **Polysaccharides**  
Neutral/charged, linear/branched, organic/inorganic substituents
- **Proteins**  
Proteins, glycoproteins, lipoproteins
- **Nucleic acids**  
DNA (eDNA), RNA
- **Lipids**  
Triacylglycerols, phospholipids, glycolipids
- **Humic substances**

# Biofouling – a special type of fouling

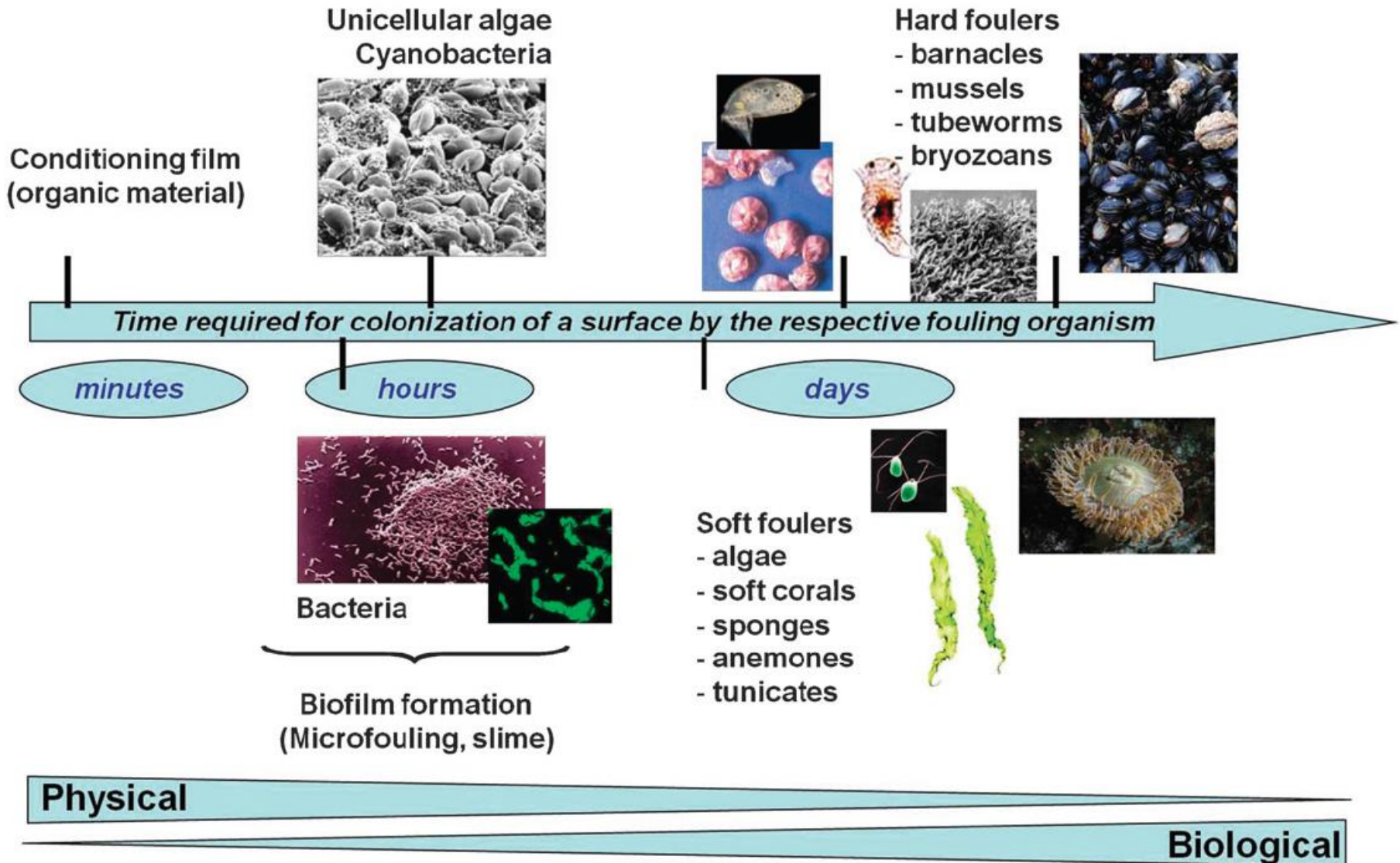
Microorganisms are living particles (biotic foulants), which can multiply in contrast to abiotic foulants.



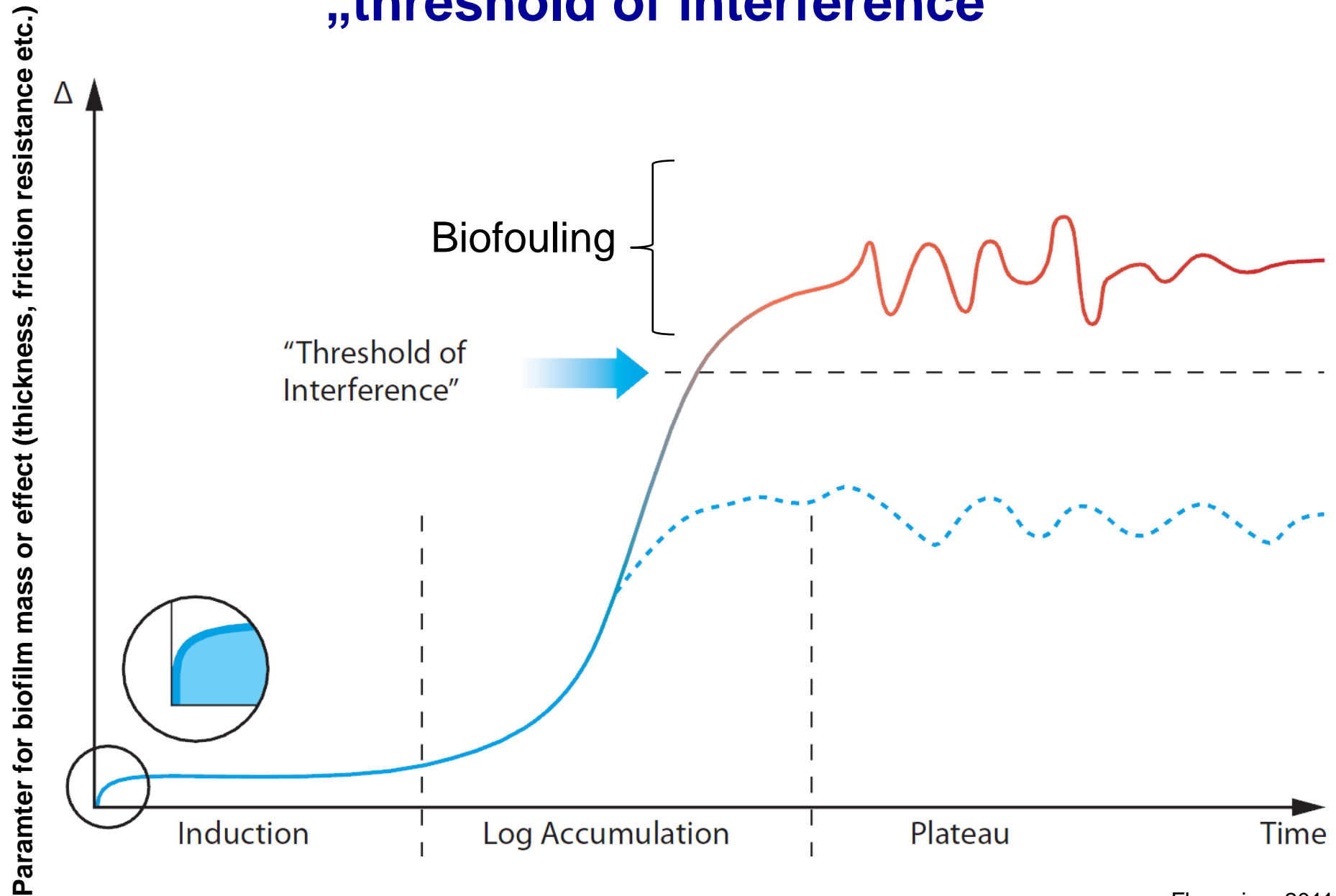
Davies, 2011

- 1: Surface colonization, reversible attachment of cells
- 2: Irreversible attachment, initiation of excretion of matrix polymers
- 3: Development of microcolonies with cells embedded in a gel-like matrix
- 4: Maturation of biofilm to high cell densities
- 5: Local dispersal of biofilms, release of biofilm cells.

# Biofouling progression over time



# Biofilm development below and above the „threshold of interference“



# Examples for technical processes in which biofouling plays a role

- Marine biofouling (ships, oil platforms, moorings etc.)
- Heat exchanger systems
- Oil production, kerosene tanks, hydraulics
- Process water systems
  - Food industry
  - Microelectronics
  - Pharmaceutical industry
  - Automobile construction
  - Production of water-based paints
  - Paper industry
- Cooling water systems
- Drinking water systems (wells, filtration membranes, distribution systems, storage tanks, plumbing systems in buildings)

# Biofouling in marine environments



(Photo: courtesy of International Paint Ltd).

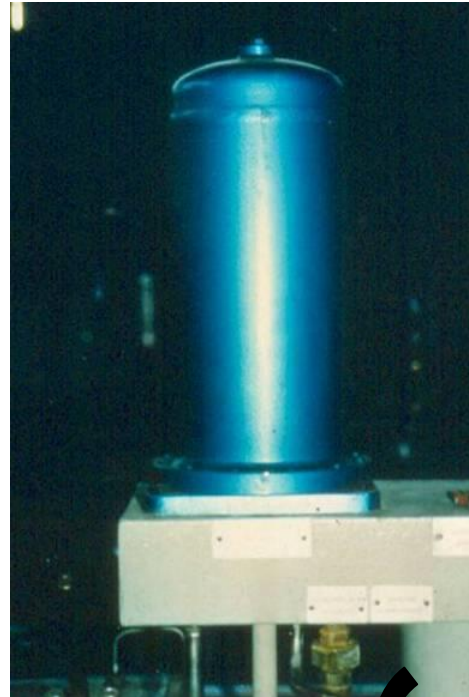


- Biofouling on ships reduces their speed (due to a reduction in hydrodynamics) and manoeuvrability, causing increased fuel and maintenance costs.
- On static structures (e.g. buoys, piers, offshore oil and gas platforms) biofouling can enhance corrosion of metal by seawater, increasing the risk of mechanical failure.
- Blooms of algae can block both fresh and salt water filtration systems, and require water pipes to be frequently cleaned to prevent blockage.

# Biofouling in heat exchangers



Heat exchangers with massive biofouling



Biofouling: a serious problem for cooling cycles in energy industry

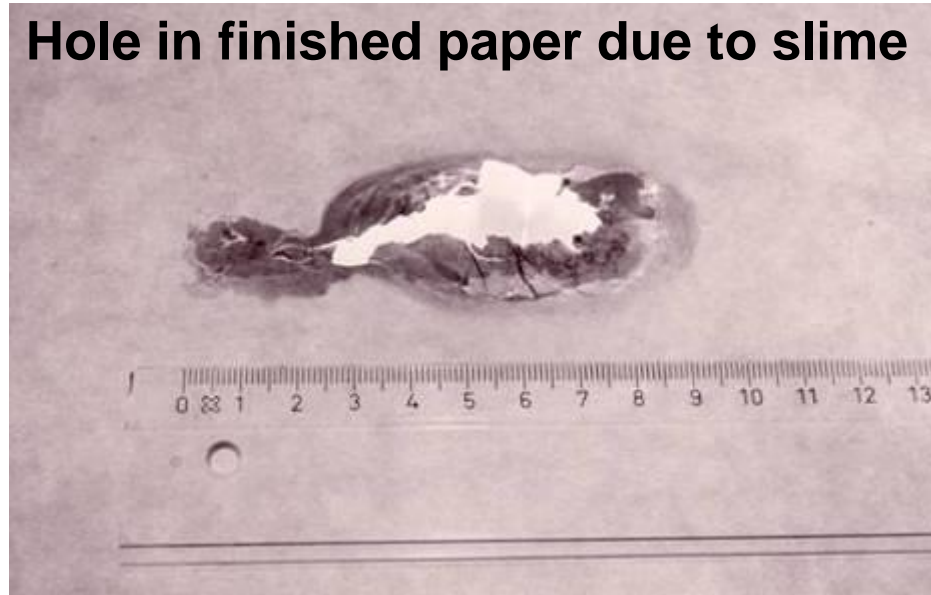
- Heat transfer limitation
- Biocorrosion



Cracking of support construction due to static overload by excessive biomass



# Biofouling in paper production



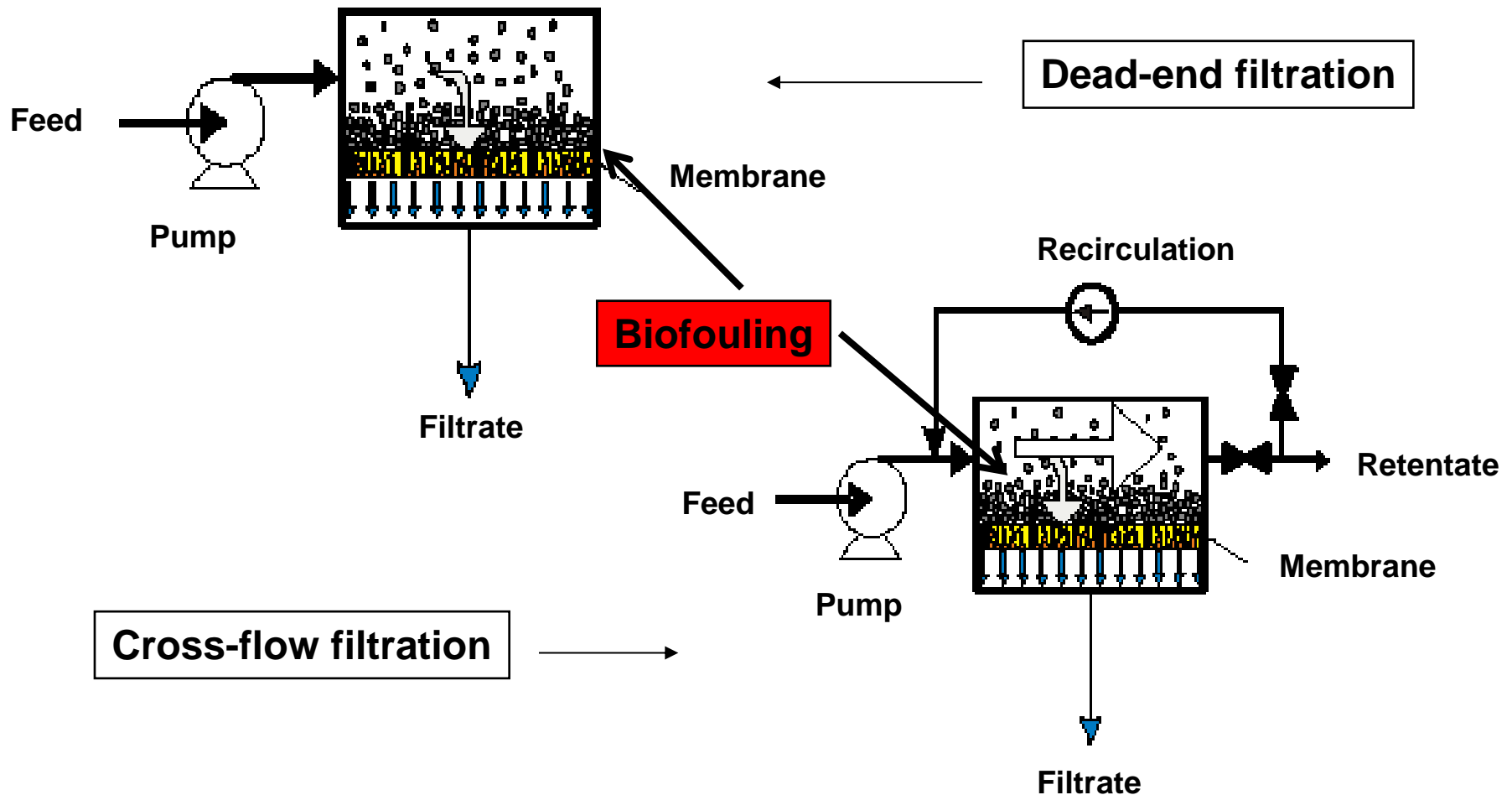
**A biofouling-friendly environment: free access of bacteria from air, water and paper components**

Biofouling chances increased by

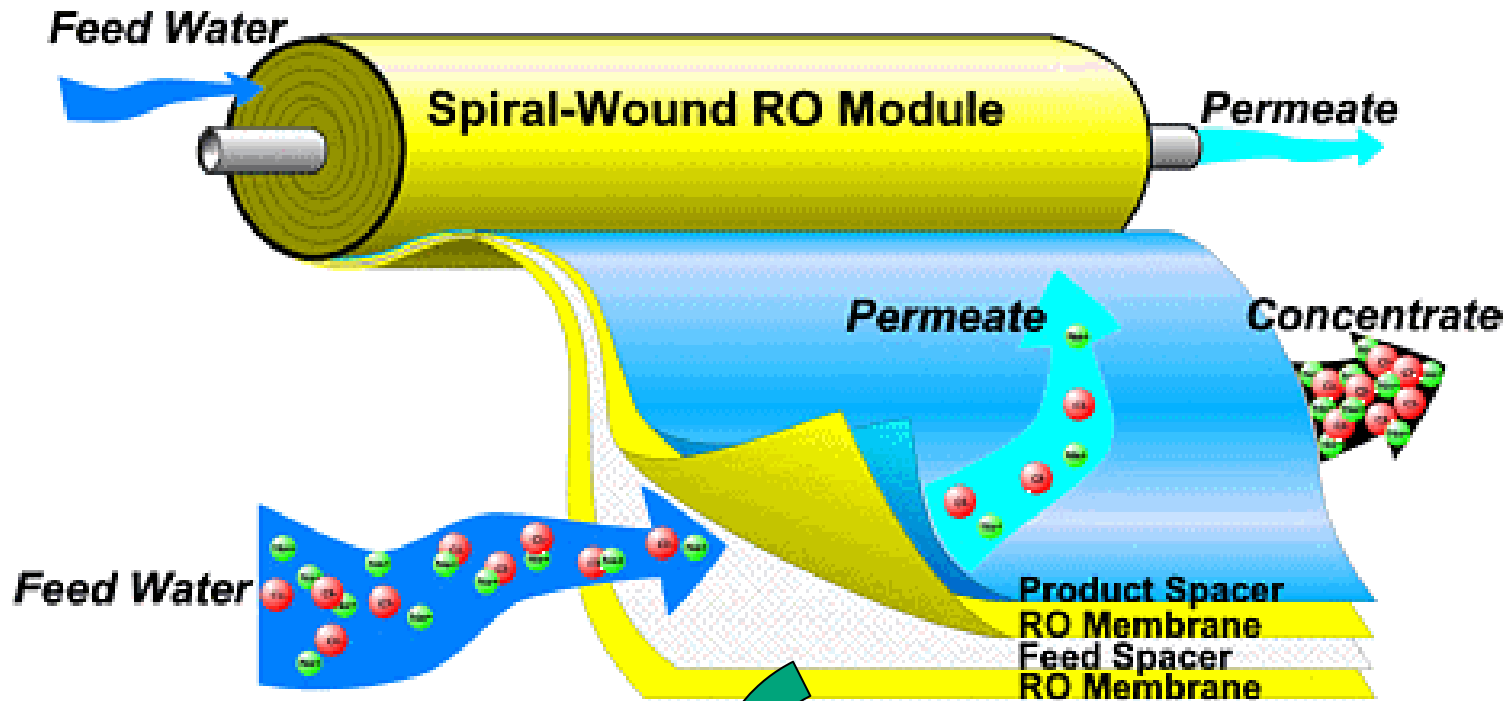
- chlorine-free process
- neutral pH-value
- elevated concentrations of nutrients
- temperatures between 30 °C and 45 °C
- use of contaminated additives, recycled paper

# Biofouling in membrane filtration

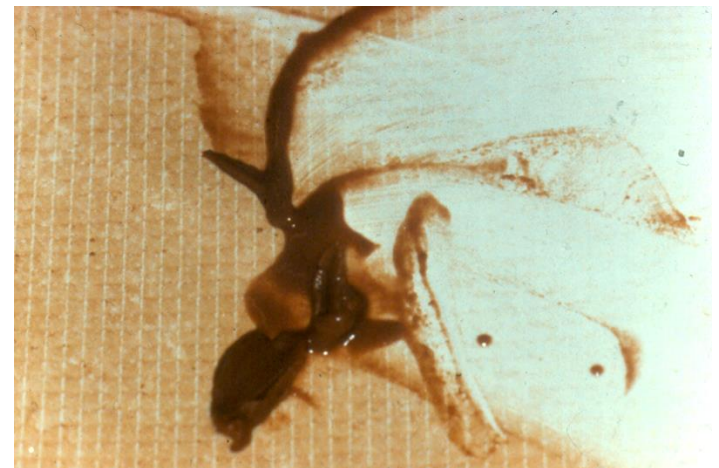
- Membrane filtration: physical separation process.
- Water is passed through a membrane, which retains particles and substances with different sizes depending on the type of membrane.

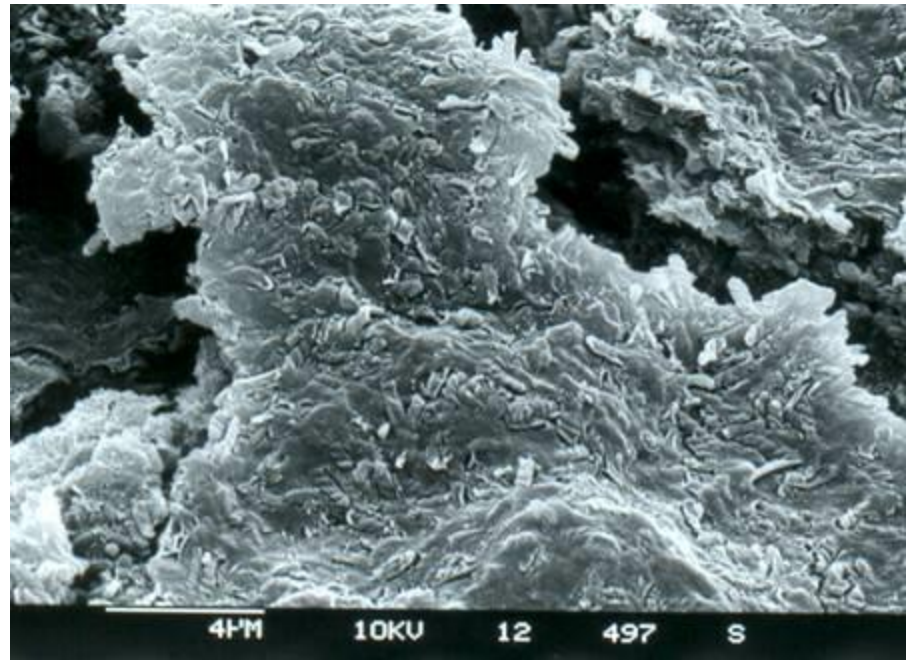
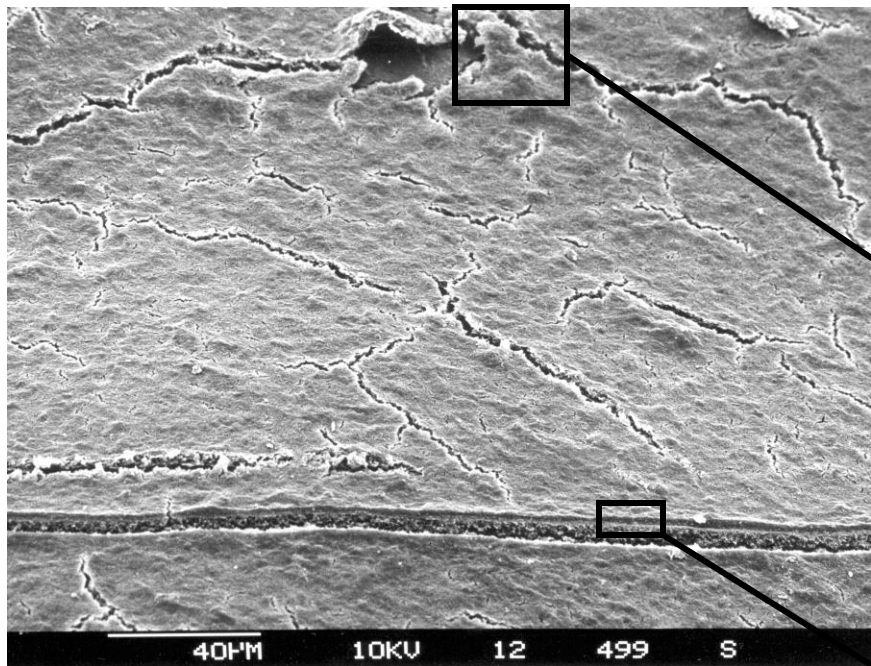


# Biofouling of reverse osmosis (RO) membranes



Biofouling of RO membrane



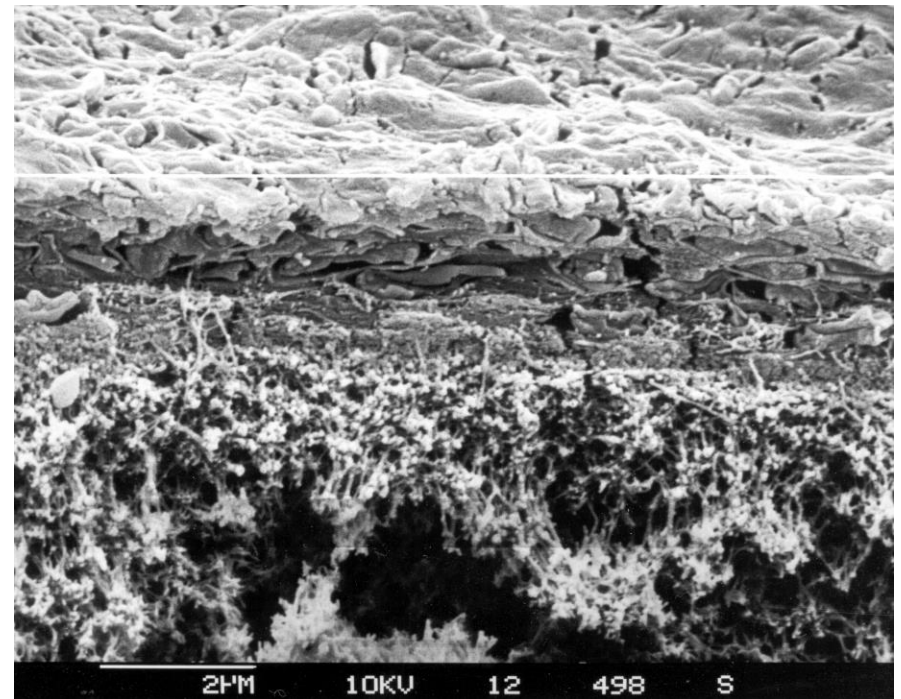


**Biofilm on an irreversibly blocked reverse osmosis membrane used for surface water treatment**

***Has survived hundreds of cleaning and disinfection cycles***

**Bacteria completely embedded in thick EPS matrix**

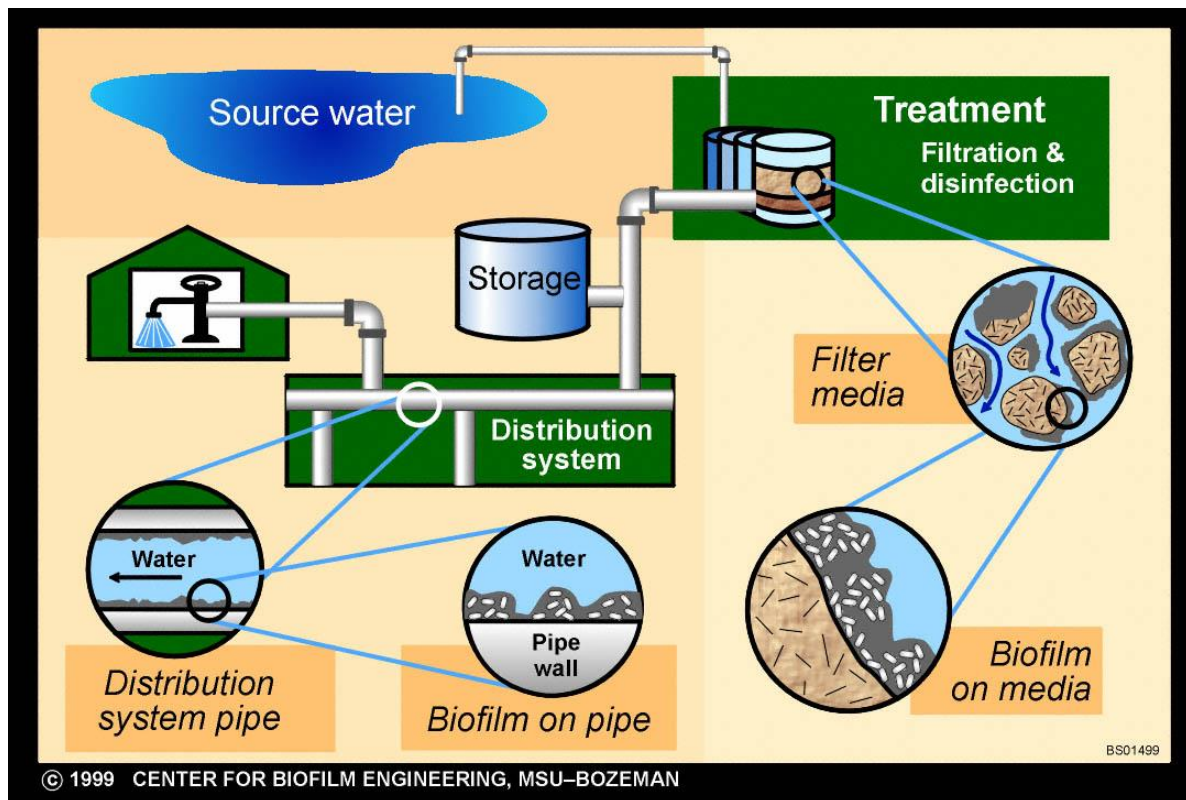
Scanning electron micrographs



# Biofilms/biofouling in drinking water systems

**Water abstraction:** internal surfaces of wells, pipes, etc. **(undesirable)**

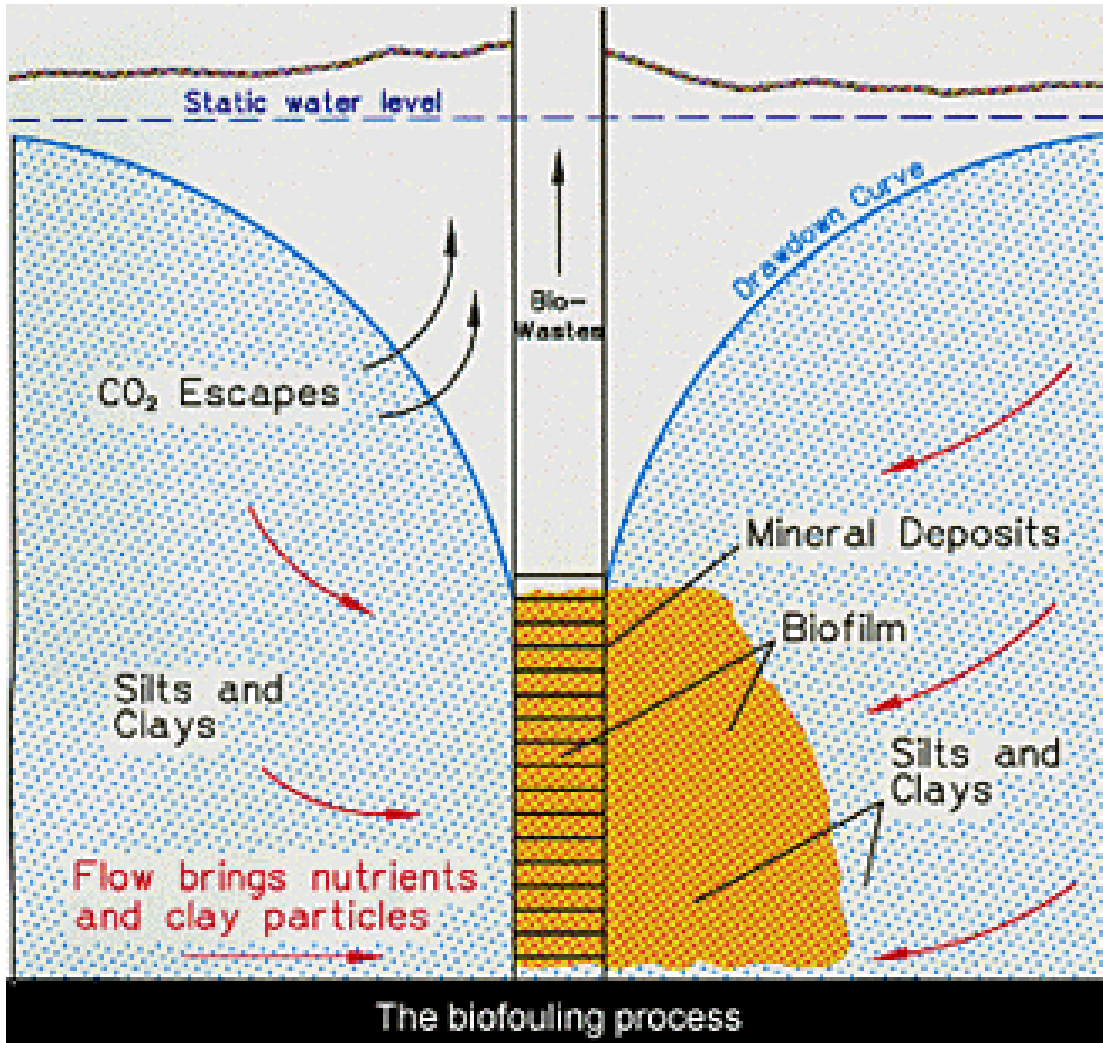
**Treatment:** surfaces of filter material (sand, activated carbon) **(desirable; removal of biodegradable substances)**, on filtration membranes **(undesirable)**



**Distribution (mains, plumbing systems):** internal surfaces of pipes consisting of mineral, metallic and non-metallic materials, tubes, etc. **(undesirable)**

**Reservoirs:** walls, floors, ceiling, calcium carbonate surface layers **(undesirable)**

# Biofouling of wells and pumps in water abstraction

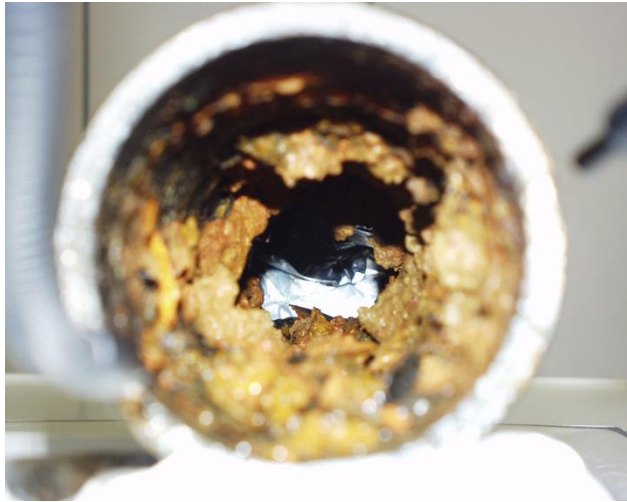


Ochreous deposits on pump.  
Source: B. Dericks

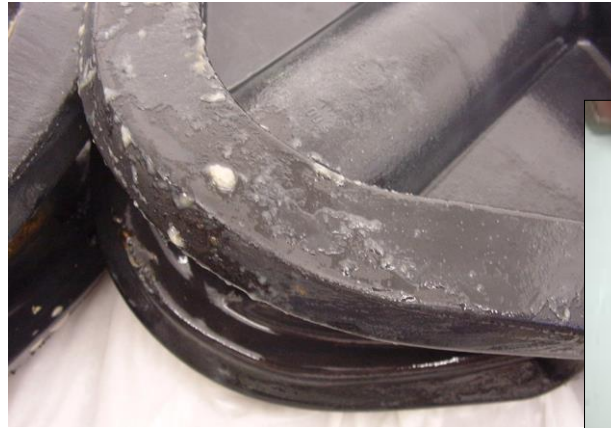
# Biofouling problems in drinking water systems

- Colonization of surfaces by hygienically relevant microorganisms, contamination of water by biofilm microorganisms (regrowth, release of microorganisms with pathogenic properties).
- Enhanced tolerance of pathogens in biofilms against disinfectants, consumption of disinfectants and formation of disinfection by-products.
- Formation of odours (e.g., caused by actinomycetes).
- Source of discoloration and turbidity of drinking water (e.g., caused by iron and manganese oxide depositing bacteria).
- Microbially influenced corrosion (biocorrosion).
- Increase in flow resistance.

# Biofouling in drinking water systems



99 year-old cast-iron pipe



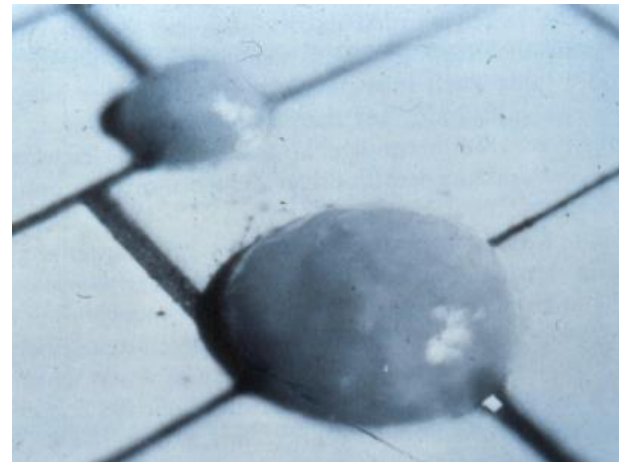
Rubber-coated valve



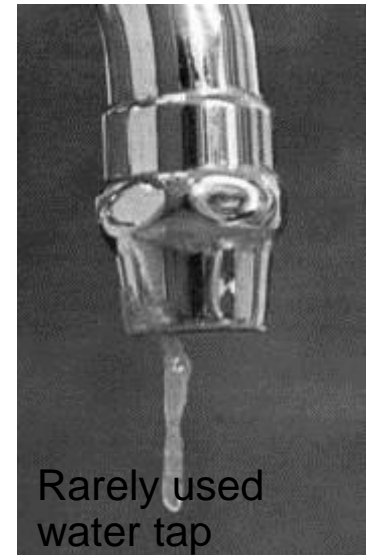
Biofilm on rubber material  
(Szewzyk, TU Berlin)



Biofilm formation on concrete wall  
of a water storage tank  
(Herb et al., 1999)



Macrocolonies between tiles  
of a water storage tank  
(Schoenen)



Rarely used  
water tap  
(Exner and Tuschwitzki, 1984)

# Biofouling in health-care facilities

Example: biofilm on a copper pipe distributing aldehyde-containing disinfectant

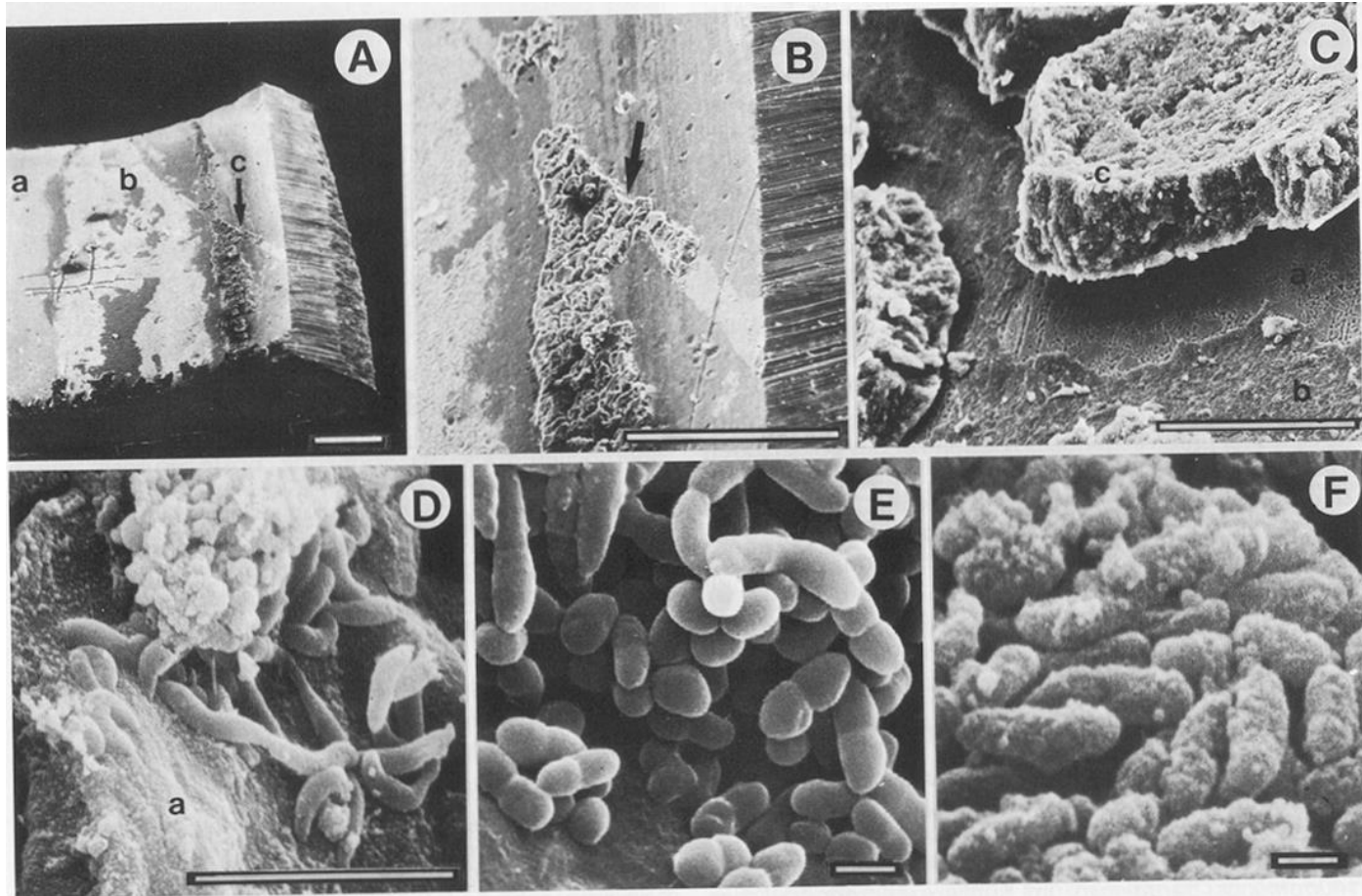


Abb. 3 Belag an der Innenwandung des Kupferrohres aus einer zentralen Desinfektionsmitteldosieranlage (REM-Aufnahmen)  
 A) Übersichtsaufnahme eines Teilstücks des Rohres: Die Orientierung des Rohres wird durch den weißen Pfeil angegeben; bei ‚a‘ ist die freie Kupferoberfläche zu sehen, bei ‚b‘ befinden sich Reste des teilweise abgehobenen Belags und bei ‚c‘ der Belag; Länge des Balkens = 1 mm  
 B) Belag auf Kupferoberfläche: Die Vergrößerung des auf der Abbildung A durch einen Pfeil markierten Bereiches läßt bei ‚B‘ die Verteilung des Belags, von dem sich Teile bereits abgelöst haben, deutlich werden; Länge des Balkens = 1 mm  
 C) Übersichtsaufnahme des Belags: Während der Probenpräparation für die REM-Untersuchung kommt es zur Austrocknung des Belags, dieser schrumpft, reißt auseinander und hebt sich schließlich von der Kupfer-

oberfläche ab: ‚a‘ freie Kupferoberfläche mit Korrosion, ‚b‘ Reste von unvollständig abgehobenem Belag, ‚c‘ abgehobenes Stück des Belags; Länge des Balkens = 0,1 mm  
 D) Unterste Schicht des Belags: Bei ‚a‘ ist die nicht-zelluläre Schicht zu erkennen, darüber sind Bakterien zu sehen, die teils frei vorliegen, teils bedeckt sind; Länge des Balkens = 10  $\mu\text{m}$   
 E) Bakterien aus dem Innern des Belags: Die glatte Oberfläche der Mikroorganismen weist auf das Fehlen einer Bekapselung hin; Länge des Balkens = 1  $\mu\text{m}$   
 F) Bakterien aus der obersten Schicht des Belags: Die raue Oberfläche der Mikroorganismen weist auf die Ausbildung von Kapselsubstanzen hin; Länge des Balkens = 1  $\mu\text{m}$ .

# Conventional anti-fouling measures

**Detection**

**Sanitation: biocides, cleaning**

**Prevention, control**

# Detection of biofouling

**Diagnosis of „biofouling“ must be confirmed.**

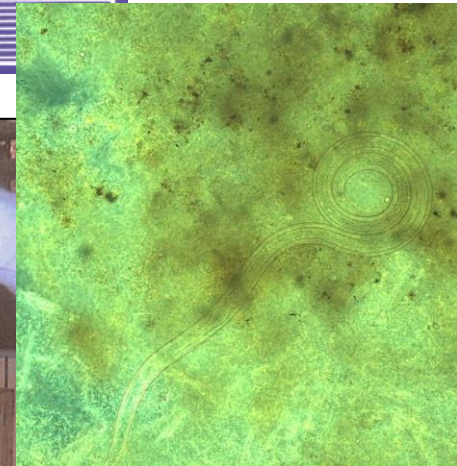
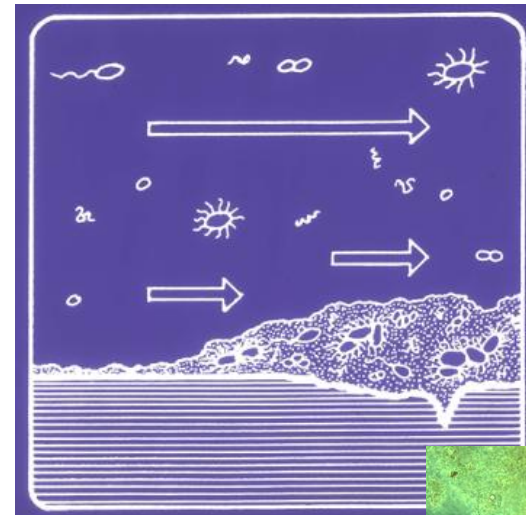
Sampling of water phase gives no indication about site and extent of biofilm formation. For localization of biofouling hot spots in large water systems: selected water samples may be useful.

## ➤ **Field methods:**

- Slime
- Smell
- Colour and consistency

## ➤ **Laboratory methods:**

- Optical inspection, microscopy
- Removal of deposit from defined surface area:
  - ⇒ Contact plate for culturing
  - ⇒ Swab method
  - ⇒ Scratching
  - ⇒ Ultrasonic treatment
  - ⇒ Glass bead abrasion



# Biofilm questions

- Location (prerequisite for analysis)
- Quantity (thickness, distribution)
- Composition (microbiological, chemical)
- Properties (physical, physiological)
- Kinetics (adhesion, growth)
- Success of countermeasures

## **Analytical laboratory methods (microbiological, chemical, microscopic, molecular)**

- Determination of biomass (viable counts, cell numbers, etc.)
- Determination of protein and carbohydrate content
- Determination of ATP content
- Determination of inorganic components
- Microscopy (light microscopy, scanning electron microscopy, confocal laser scanning microscopy)
- Determination of biofilm thickness

# Antifouling measures: The medicine-based strategy

- Biofouling is considered a „technical disease“.
- Microorganisms cause this disease.
- Kill microorganisms and the problem is solved.
- Adoption of medical concept: „disinfection“ to cure the system.

Drawback of this strategy:

- In many cases ineffective, because biomass, not activity is the problem, dead or alive.
- Killing does not necessarily remove biomass: dead biomass can still cause problems (heat exchanger, membranes, process waters, drinking water, etc.).
- „Disinfection“ means only „inactivation of pathogenic organisms“.

# What is a biocide/biocidal product?

Definition of a biocidal product according to the EU Biocidal Products Directive:

**"Active substances and preparations containing one or more active substances, put up in the form in which they are supplied to the user, intended to destroy, deter, render harmless, prevent the action of, or otherwise exert a controlling effect on any harmful organism by chemical or biological means."**

Biocide is a generic term and comprises

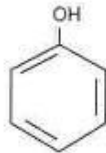
- microbicides (bactericides, fungicides, algicides, etc.), disinfectants
- molluscicides
- insecticides
- herbocides
- rodenticides, etc.

# Oxidizing biocides

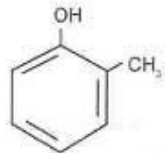
- **Chlorine**
- **Chlorine dioxide (ClO<sub>2</sub>)**
- **Ozone (O<sub>3</sub>)**
- **Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>)**
- **Potassium permanganate (KMnO<sub>4</sub>)**
- **Bromine**
- **Iodine**

# Non-oxidizing biocides

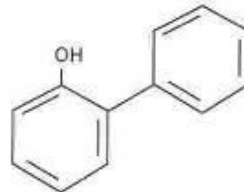
## Phenolics



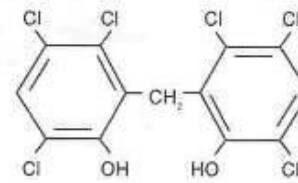
Phenol



Orthocresol

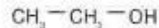


Orthophenylphenol

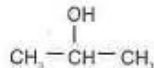


Hexachlorophene

## Alcohols

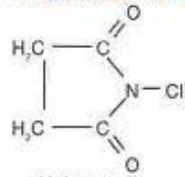


Ethanol



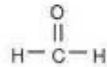
Isopropanol

## Halogenated compound

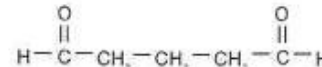


Halazone

## Aldehydes

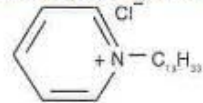


Formaldehyde

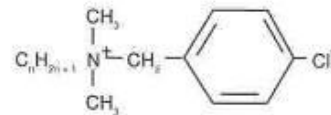


Glutaraldehyde

## Quaternary ammonium compounds

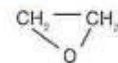


Cetylpyridinium chloride

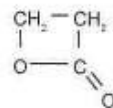


Benzalkonium chloride

## Gases



Ethylene oxide



Betapropiolactone

**Figure 7.7 Disinfectants and Antiseptics.** The structures of some frequently used disinfectants and antiseptics.

# Factors that determine the survival of microorganisms in the presence of biocides

- Type and state of microorganism (resistance properties)
  - group and physiological state of microorganism
  - physical state (planktonic, cell aggregates, biofilms)
- Choice of biocide
- Contact time
- Concentration of biocide
- Abiotic factors
  - water temperature
  - pH value
  - redox potential
  - turbidity
  - inorganic and organic substances

# Inactivation: biocide concentration and contact time

The relationship between biocide concentration and contact time is given by the Chick-Watson law:

$$K = C^n \cdot t$$

K: constant for a given microorganism exposed to a disinfectant under specific conditions

C: residual disinfectant concentration (mg/L)

t: time required to kill a certain percentage of the population (min)

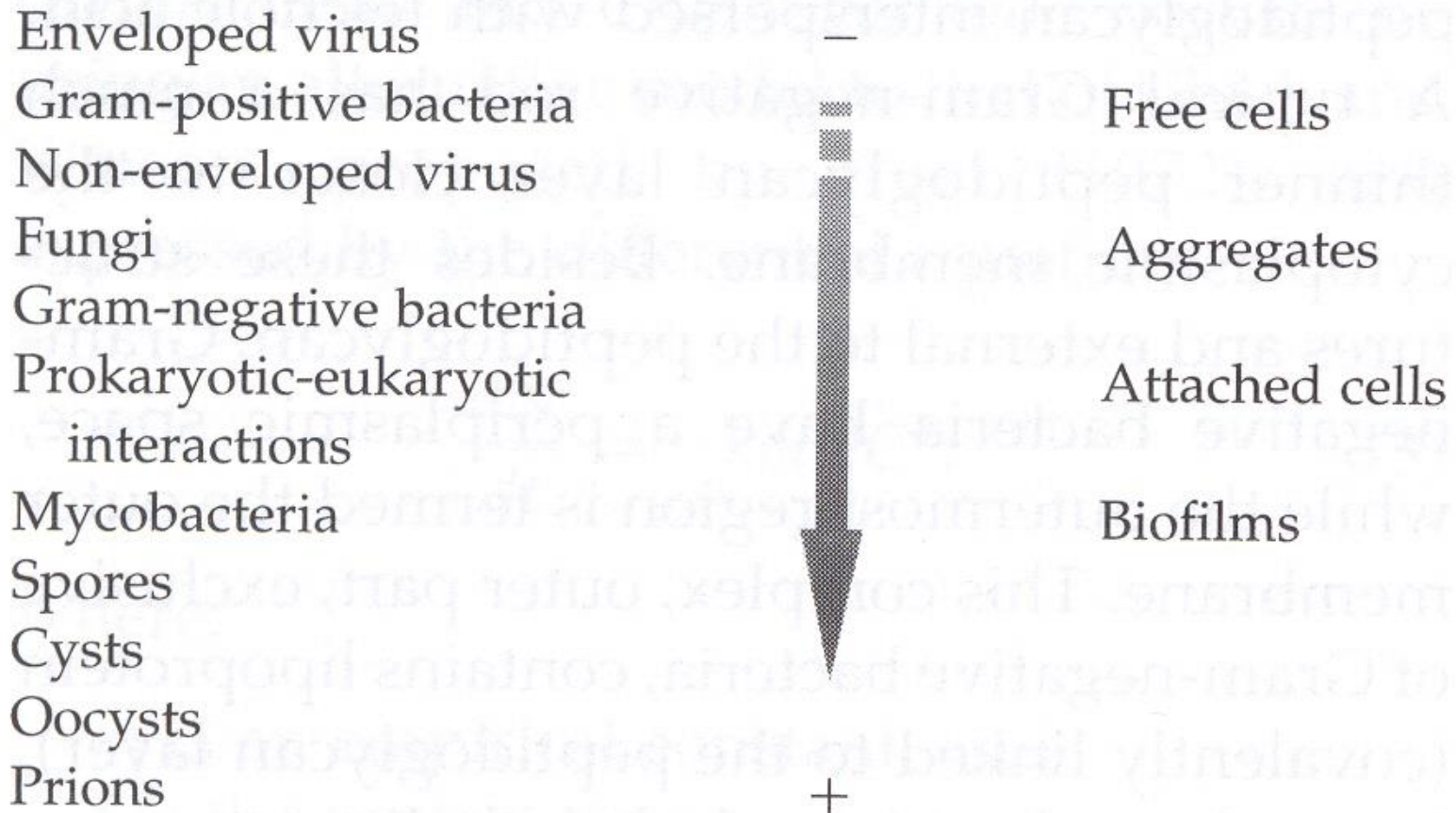
n: constant (coefficient of dilution); in most cases n equals 1  $\Rightarrow$  disinfection effectiveness may be expressed as Ct

**TABLE 6.1. Microbial Inactivation by Chlorine: Ct Values (Temperature = 5 C°; pH = 6.0)**

Microorganism	Chlorine Concn (mg/L)	Inactivation Time (min)	Ct
<i>Escherichia coli</i>	0.1	0.4	0.04
Poliovirus I	1.0	1.7	1.7
<i>Entamoeba histolytica</i> cysts	5.0	18	90
<i>Giardia lamblia</i> cysts	1.0	50	50
	2.0	40	80
	2.5	100	250
<i>Giardia muris</i> cysts	2.5	100	250

Adapted from Hoff and Akin, 1986.

# Relative microbial susceptibility to chemical biocides

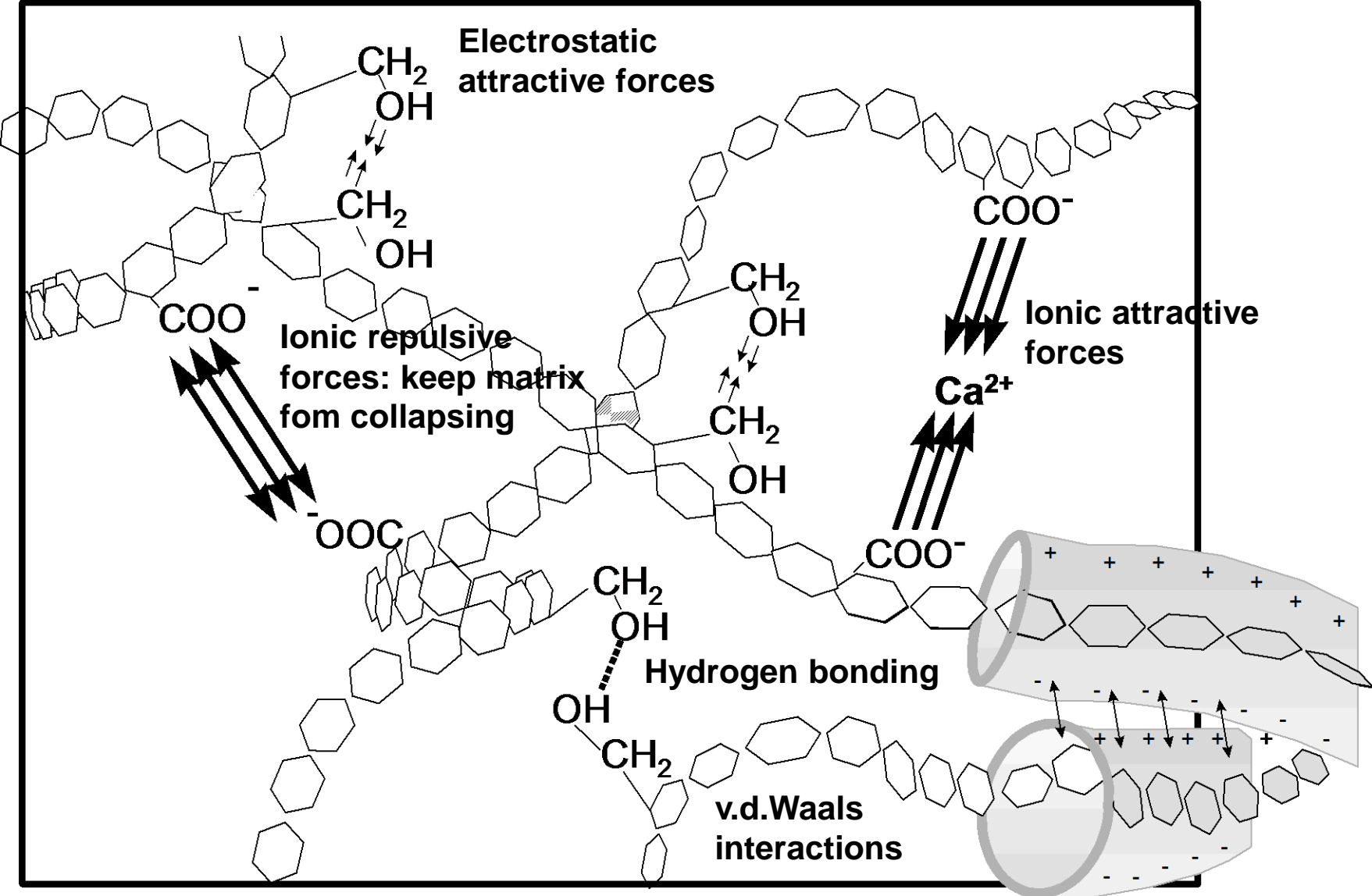


-, least resistant  
+, most resistant

# What is cleaning?

- Cleaning is the removal of (bio)fouling layers by physical and/or chemical means.
- The most important task is the de-attachment of the foulant from the surface to be cleaned.
- A dissolution of the foulant is not absolutely required.
- The de-attached foulant has to be removed.
- In cleaning solutions for multiple use the de-attached foulant has to be kept in a stable suspension in order to prevent redeposition on the cleaned surfaces.

# Weak interactions keep biofilms together



# A cleaner has to overcome mechanical stability of biofilms

Goal of cleaning: interference with weak interaction forces

➤ **Electrostatic interactions:**

- Complexing substances (EDTA, NTA etc.)
- Acids and bases

➤ **London interactions (hydrophobic, van der Waals)**

- Surfactants of all kind

➤ **Hydrogen bonds**

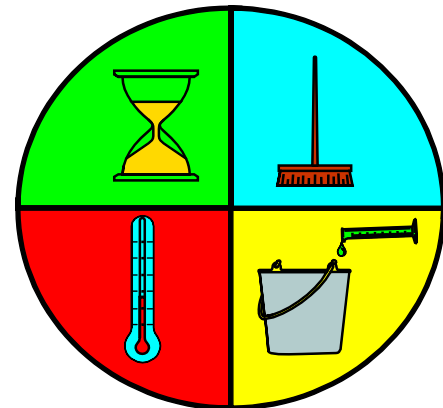
- Chaotropic substances (urea, methyl urea etc.)

➤ **Covalent bonds of biopolymers:**

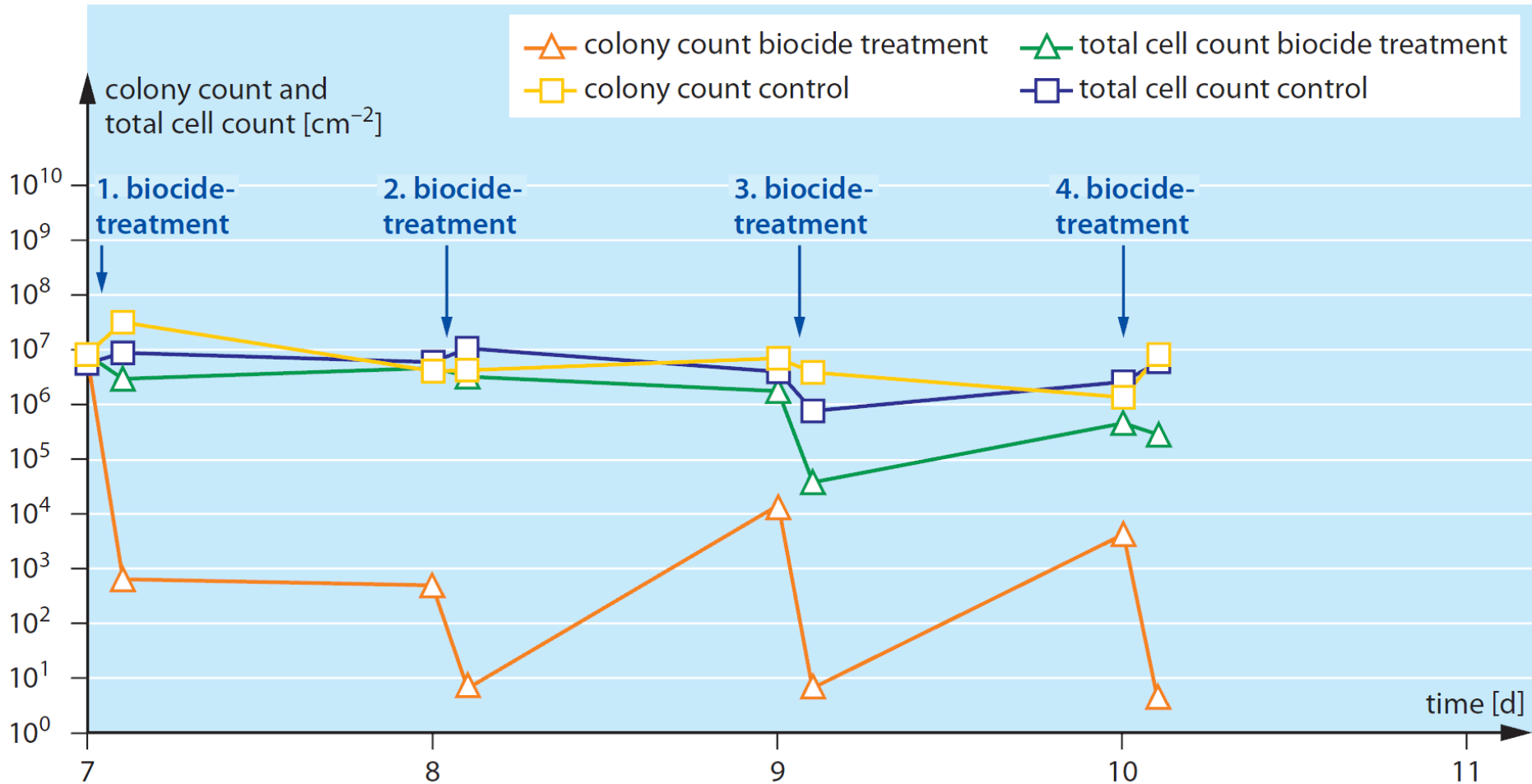
- Oxidizing agents (chlorine, ozone, hydrogen peroxide)
- Enzymes: proteases, amylases, pectinases, lipases, mixtures

➤ **Parameters relevant for efficacy:**

- Exposure time
- Concentration
- Temperature
- Mechanical energy



# Killing is not cleaning!



Repeated treatment of mixed-population biofilm with  $\text{H}_2\text{O}_2$  and peracetic acid (28 ppm/1.2 ppm) for 1 hour.

(Schulte, 2003)

⇒ Biocide application **and** cleaning are important, because dead biofilm mass serves as a nutrient source for newly colonizing microorganisms!

# Most effective cleaning: mechanical

Mechanical removal (e.g., high-pressure cleaning, air-water flushing, flexible brushes for pipe systems); efficient method in many applications.

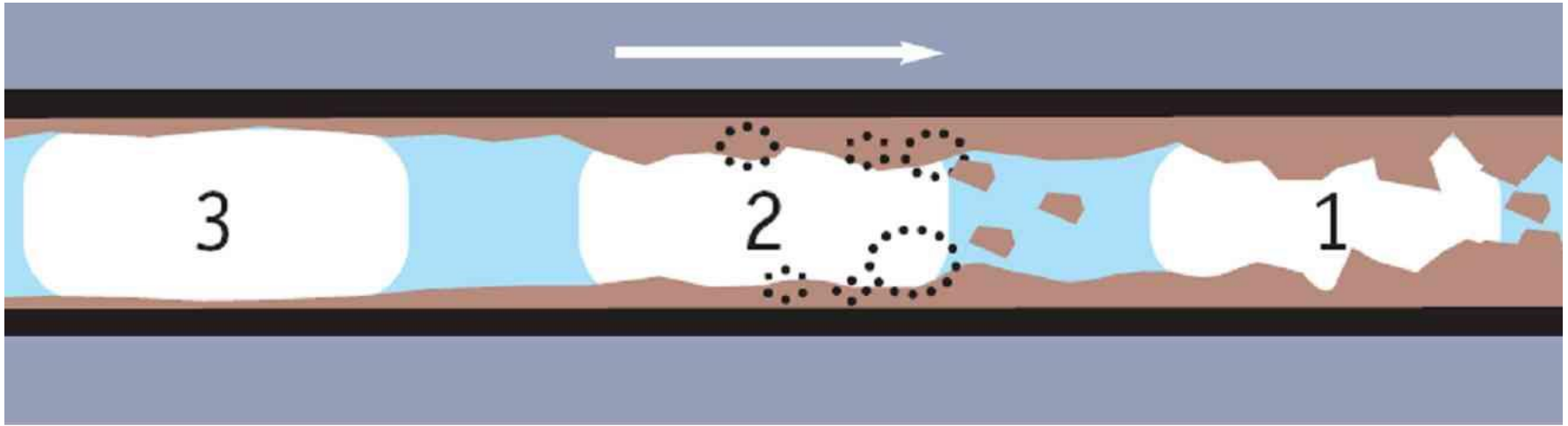


Pipeline engineering manufactures a wide range of pigging, joint testing and isolation equipment



Pigging of pipelines („Molchen“)

# Air-water flushing („Impuls-Spülverfahren“)



„Impuls-Spülverfahren“: Using the mechanical shear forces of water and air bubbles

- more effective than flushing only
- saves water



## Five expensive mistakes in conventional anti-fouling strategies:

- ***No early warning systems*** (monitoring by process performance or product quality)
- ***No information on biofilm site/extent*** (sampling of the water phase and not on surfaces)
- ***„Disinfection“ is not cleaning*** (Biocides leave dead biomass on surface, good regrowth)
- ***No nutrient limitation*** (nutrients = potential biomass; not reduced by biocides)
- ***No optimization of countermeasures*** (efficacy control only by process or product quality)

# Biofilm Management

Keep biofilm growth below „threshold of interference“

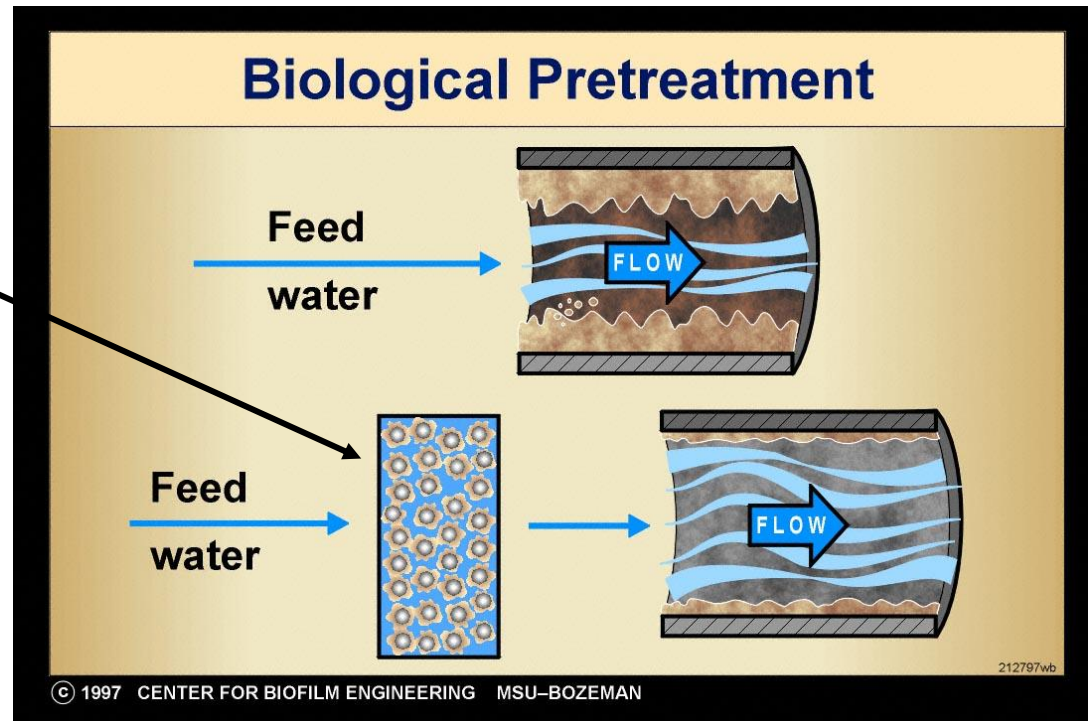
## Some tools

- **Monitoring**
- **Fouling factor analysis**
- **Use of low-fouling surfaces**
- **Nutrient limitation**
- **Limitation of access of microorganisms**
- **Growth inhibition (“biocides”)**
- **Strategic cleaning**
- **Destabilization of biofilms**
  - Shear forces
  - Mechanical cleaning
  - Chemical cleaners

# Control of biofilm formation in water systems

- Minimization of nutrients
  - removal of microbially utilizable substances during water treatment (aim: biologically stable water),

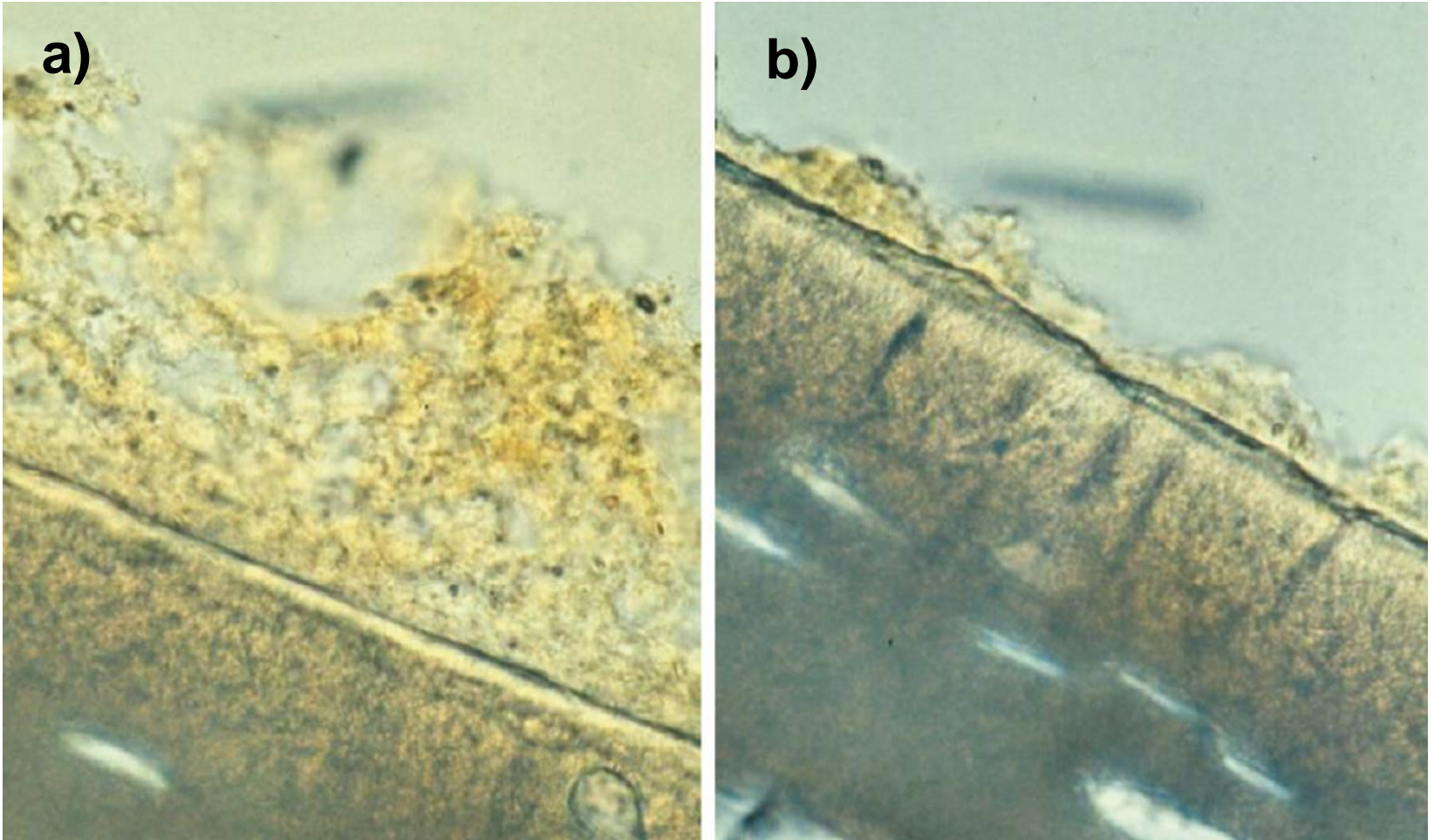
For example, sand filters or activated carbon filters (biofilm reactors) removing biodegradable compounds



- materials which do not release biodegradable substances.
- Low-fouling surfaces.
- Removal of microorganisms during water treatment.
- Biocide application.

# Example: Control of biofilms in membrane systems

Limitation of biofilm growth to prevent biofouling by sand filters (“biofilm reactors in the right place”) placed ahead of membrane system.



Biofilm on a reverse osmosis membrane (a) before sand filter, (b) after sand filter. Magnification: 400-fold. (Griebe and Flemming, unpublished)

# Approaches to minimize primary adhesion of organisms by surface modifications

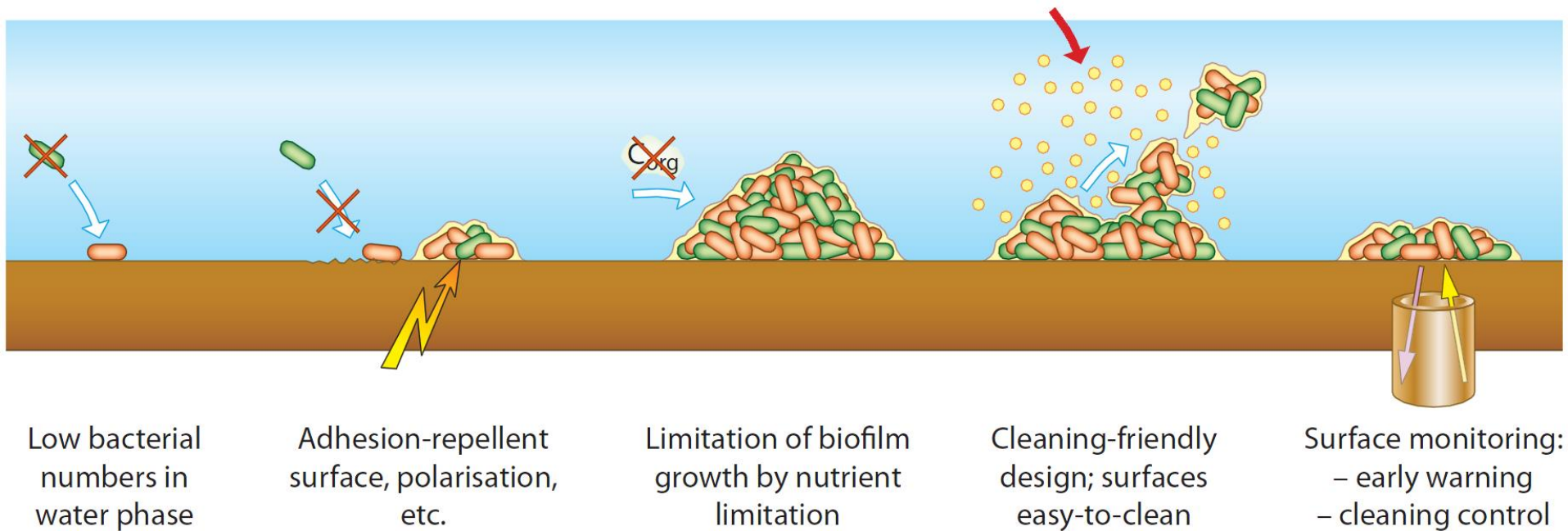
**Strategy:** Selection or development of surfaces which are not readily colonized by microorganisms and are easy to clean (**low-energy surfaces**)

## Examples

- Smoothing of surfaces
- Superhydrophilic surfaces
- Superhydrophobic surfaces
- Microstructured surfaces
- UV-activated TiO<sub>2</sub> coatings, Ag nanoparticles
- Pulsed surface polarization, pulsed electrical fields
- Low-surface energy coatings
- Incorporation of antimicrobials
- Biocides directly generated on surfaces
- Surface-bound biocides and antimicrobial peptides
- Combined, multiple approaches

# Integrated, holistic anti-fouling strategy

- Biofilms cannot be eradicated forever.
- Successful strategies against biofouling should be based on integrated approaches, which consider the entire system to be protected.



Key elements of an integrated antifouling strategy (Flemming, 2011)