Biofilm Growth and Disinfection in Water Systems

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Topics

- Biofilm basics
- Why biofilms are hard to kill
- Visualizing antimicrobial action against biofilms
- Heat inactivation of biofilms
Biofilm Viewed with Confocal Microscopy
Time-lapse confocal imaging

Green is gfp P. aeruginosa PA01
Red is dsRed E.coli 0157:H7


Ben Klayman
Potable water biofilms
Why Biofilms Are Hard to Kill:
Biofilm Tolerance to Antimicrobial Agents
Biocide Effect on Planktonic Cells and Biofilm

*P. aeruginosa* – Glutaraldehyde (50 mg/l)
Antibiotic Effect on Planktonic Cells and Biofilm

*P. aeruginosa* – Tobramycin

![Graph showing time (h) vs. Log (X/X₀) for control, biofilm, and planktonic states.](image)

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Biofilm Tolerance of Antimicrobial Agents

Slow Penetration
Hydrogen Peroxide fails to Penetrate Biofilm

H$_2$O$_2$ \xrightarrow{\text{catalase}} H$_2$O + $1/2$O$_2$

Bulk fluid

Base of biofilm

Time (seconds)

Hydrogen Peroxide Concentration (mM)
Chloride Ion Penetrates Biofilm Readily
…but reactive Hypochlorite Ion does not
Chlorine Penetrates Biofilm Slowly

![Graph showing chlorine concentration over depth into biofilm (microns)].

- Chlorine Concentration (mg/L)
- Depth into Biofilm (microns)

Key data points:
- 5460 s
- 1511 s
- 47 s

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Biofilm Tolerance of Antimicrobial Agents
Growth Activity in an Aerobic Biofilm measured using GFP Expression in *P. aeruginosa*
Oxygen Penetration into *P. aeruginosa* Biofilm

**Graph Representation**

- **Y-axis**: Oxygen Concentration (mg/l)
- **X-axis**: Depth (μm)

**Legend**

- **sterile control**
- **untreated**

**Data Points**

- Oxygen concentration decreases as depth increases.
- The sterile control shows a higher oxygen concentration near the surface compared to the untreated sample.

**Observations**

- The untreated sample exhibits a rapid decrease in oxygen concentration, indicating lower oxygen penetration into the biofilm.
- The sterile control maintains a higher oxygen concentration over a greater depth, suggesting a more effective barrier against oxygen penetration.

**Implications**

- Understanding oxygen penetration into biofilms is crucial for designing effective treatments and therapies.
- The differences in oxygen penetration between sterile controls and untreated samples highlight the impact of biofilm formation on environmental conditions such as oxygen availability.

**Conclusion**

Further research is needed to explore the mechanisms behind these observations and to develop strategies to enhance oxygen penetration into biofilms for improved biofilm control.
S. epidermidis biofilm staining with Calcein AM green (CAM)

Time: 1 hr

Vel = 6 cm/sec
CAM-stained *S. epidermidis* biofilm treated with 10 mg/L sodium hypochlorite

Time: 1 hr

Vel = 6 cm/sec
Biofilm control: 50 mg/l Hypochlorite

CAM-stained *S. epidermidis* biofilm treated with 50 mg/L sodium hypochlorite

Time: 1 hr

Vel = 6 cm/sec
Biofilm control: 50 mg/l Hypochlorite

CAM-stained *S. epidermidis* biofilm treated with 50 mg/L sodium hypochlorite

Time: 1 hr

Vel = 6 cm/sec
Treatment with 50 mg/l quat

Biofilm ripple “creep”

Run time: 12 hr
Re= 3500

Biofilm Detachment

Stoodley, P., Wilson, S. & Costerton, J.W.
Options for Microbial Control

- Stop Attachment
- Stop Growth
- Block Matrix Synthesis
- Kill
- Disrupt Communication
- Promote Detachment
- Mechanical Removal
Biofilm Control in Water Systems

- Regular cleaning and antimicrobial dosing
- Antimicrobial access to the biofilm
- Contact time
- Materials Compatibility
- Measuring successful treatment
Industrial Associates Program

Program Benefits:

- Montana Biofilm Meetings
- Workshops
- Consulting/Strategic Planning
- Research/Testing Projects
- Regulatory Interactions
- Connection to the Biofilm Community
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**Energy**
- ExxonMobil
- BP

**US Gov’t Programs/Labs**
- NASA