Fundamentals and Applications of Biofilms
Bacterial Biofilm Formation and Culture

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Introduction

- Environmental factors: pH, Temp, nutrients
- Microorganisms in the soil, water and air.
- Microbial activity at interfaces: between solid and liquid phases.
Microbial Attachment (Adsorption, Adhesion)

**Definition:** intimate interaction of a cell with the substratum
Relevance of Biofilms

Detrimental effects
- Increase the transfer resistance of mass, heat and momentum
- Deteriorate attached materials due to biological and chemical reactions
- Health risks

Beneficial effects
- Increase biomass concentration
- Facilitate biomass-liquid separation
- Improve overall productivity
Biofilm Formation Process

1. Transport
2. Duplication
3. Extracellular polymer production
4. Lysis
5. Attachment
6. Duplication
7. Detachment
Biofilm animation 1, 2
Biofilm Formation Process

In liquid phase
- Dissolution of organic macromolecules
- Pre-conditioning adhesion
- Cell transport
- Replication
- Production of extracellular polymers
- Death
- Attachment to the substratum

Within biofilms
- Replication
- Production of extracellular polymers;
- Detachment
Biofilm Formation Process

Pre-conditioned film
- Organic molecules transported from the bulk fluid to the substratum, where some of them adsorbed, resulting in conditioned substratum.

Cell transport
- Planktonic cells transported to the conditioned substratum.

Biofilm Formation Process

Adsorption

Some cells adsorbed to the substratum for a while and then desorb due to biological, chemical and physical factors (reversible adsorption); some remained immobilized (irreversible adsorption).

Biofilm Formation Process

Growth
Extracellular polymer production

Detachment

Net Biofilm Formation

Biofilm net accumulation = Deposition rate from liquid + Growth rate phase - Detachment rate

\[ \frac{dB}{dt} = k_{att} X + \mu B - k_{det} B \]
Net Biofilm Formation

Deposition (Attachement)

Total adsorbed cells = reversibly adsorbed cells + irreversibly adsorbed cells

Sticking efficiency = \( \frac{\text{No. of cells adsorbed to the substratum}}{\text{No. of cells transported to the substratum}} \)

Transport velocity, concentration

Adsorption velocity, concentration, physical, chemical and biological properties.
Net Biofilm Formation

Growth

\[
\text{Growth rate (\(\mu\))} = \frac{\mu_m \ S}{K_S + S} \]

\(\mu\) specific growth rate
\(\mu_m\) maximum specific growth rate
\(S\) substrate concentration
\(K_S\) saturation constant
\(B\) biofilm density
Net Biofilm Formation

Detachment

Erosion a continuous loss of small portion of biofilms.

Sloughing a rapid, massive loss of biofilms.

Abrasion due to repeated collisions between substratum and particles.

Detachment - $k_{\text{det}} \cdot B$

$k_{\text{det}}$ Shear force, roughness
Culture

Essentials of biofilm formation

- Substratum, aquatic environment, microbial cells, nutrients, attachment

Biofilms can be found everywhere in natural environment

- Mixed culture, various (uncontrolled) condition, difficulty in sampling

Provide an appropriate situation to enhance biofilm formation
Biofilm Culture Considerations

Source: www.erc.montana.edu
Experimental Systems

The reactor

Provide substratum for biofilms to accumulate.

The reactor feed

Provide an aquatic environment containing all cellular growth requirements (energy, carbon, trace elements at appropriate concentration)

Environmental control system

Provide controlled flow velocity, temperature, pH

Specific treatment

Provide specific treatment such application of antimicrobial agents or microelectrodes.
System Consideration

Accumulate maximal biofilms at minimal medium and time
Allow the least invasion
Provide the most samples
Reduce variables
Experimental Variables

Chemical
- Substrate type
- Substrate concentration
- pH
- Inorganic composition
- Dissolved oxygen
- Microbial inhibitors

Physical
- Temperature
- Fluid shear stress
- Heat flux
- Surface composition
- Surface texture (roughness)
- Hydraulic residence time

Biological
- Organism type (mixed or pure cultures; aerobic or anaerobic)
- Organism concentration
Reactor Design

Balance equation
Net rate of Accumulation = Net rate of input by transport + Net rate of production by transformation

Mass balance

- Batch culture
- Continuous Stirred Tank Reactor (CSTR); Chemostat
- Plug Flow Reactor (PFR)
Mass Balance

Batch culture

Well-mixed; No input or output (Q = 0)

\[ V \frac{dC}{dt} = V \cdot r \]

C: concentration (mass L\(^{-3}\))

r: conversion rate (mass L\(^{-3}\) time\(^{-1}\))
Mass Balance

Continuously Stirred Tank Reactor (CSTR)

Chemostat

Well-mixed; no gradient; \( Q_{\text{in}} = Q_{\text{out}} \)

\[
\frac{dC}{dt} = Q (C_i - C) + V \cdot r
\]

at Steady State

\[
\frac{dC}{dt} = 0
\]

\[
\frac{Q}{V} (C_i - C) = -r
\]

Q: flow rate (L\(^3\) time\(^{-1}\))

C: concentration (mass L\(^{-3}\))

r: conversion rate (mass L\(^{-3}\) time\(^{-1}\))

D: dilution rate (time\(^{-1}\))

\( \tau \): 1/D, residence time
\[ V \frac{dC}{dt} = Q (C_i - C) + V \cdot r \quad r = \mu X \quad \text{growth rate} \]

0 if sterile feeding

\[ \frac{dC}{dt} = \frac{Q}{V} (C_i - C) + r \]

\[ \frac{dX}{dt} = (\mu - D) X \]

\[ \text{BST} \]
Mass Balance

Plug Flow Reactor (PFR)

Not well-mixed; the composition changes with distance through the reactor

\[ \frac{\partial C}{\partial t} + V \frac{\partial C}{\partial z} = r \]

rate of conversion

\[ \frac{\partial C}{\partial t} + V \frac{\partial C}{\partial z} = D \frac{\partial^2 C}{\partial z^2} + r \]

rate of transport by diffusion
Biofilm Culture

CSTR

- Provides advantages for observing, separating and evaluating the kinetics and stoichiometry of each biofilm process.

- The bulk liquid phase is uniform

- The steady state condition is convenient and reproducible

- Constant shear stress
Biofilm Reactor

Roto Torque (Rotating Annular Reactor)

Source: BioSurface Technologies Corp.
Biofilm Reactor

Parallel plate flow cell reactor

Flow cell reactor
Biofilm Reactor System

Source: www.erc.montana.edu

Source: BioSurface Technologies Corp.
Biofilm Reactor

Tubular Reactor

Robbin’s Devices
Biofilm Reactor

Radial flow reactor

Rotating disk reactor
Eight rods that can be sampled at different times during continuous flow

Control and treated coupons in the same reactor

Twenty-four coupons for more sampling opportunities

Various coupon materials are available and are interchangeable with RDR

Baffled stir bar for consistent shear
Biofilm Reactor

Stirred disk reactor

Drip-flow plate reactor
Biofilm Reactor

Propeller reactor

Fixed film reactor