Fundamentals and Applications of Biofilms
Bacterial Biofilm Applications

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Introduction

**Water treatment**
- For limiting biological growth and biofilm formation

**Wastewater treatment**
- Remove toxic chemicals via the formation of biofilms

**Bioremediation**
- Remove or detoxify pollutants that have found their way into the environment and threaten public health

**Medical Biofilms**
- Immunology of biofilms
Water Cycle

International Water Association
Water Treatment

Bacterial growth in purified water

Growth promoting factors in purified water system
- Carbon, nitrogen, phosphorous/sulfur, trace elements and salts, light

Starvation survival
- Starvation $\rightarrow$ Tend to utilize endogeneous energy

Biofilm formation
Water Treatment

Detection methods

Deficiencies associated with cultural techniques

- In situ biofilm monitoring
- ATR-FTIR (Attenuated Total Reflectance-Fourier Transforming Infrared Spectrometry)
- Resonance Raman Spectroscopy
- Fluorometry
- Electrochemical monitoring
Water Treatment Consideration

Influence of biofilms on treatment efficacy
- Bacterial regrowth

Physical treatment
- High temperature (> 80°C)
- Stream flush
- Bioelectric field
- Ultrasonication
Why Wastewater Treatment?

Solubility of oxygen is low; DO conc. < 8mg/Liter

Aeration from atmosphere or evolution by photosynthesis is much slower than the utilization

Overload of organic components on natural waters

Lowering or exhaust the DO content.

Instead of $O_2$, Sulfate or nitrate used as electron acceptors.

Noxious odors, tastes and colors.

Anaerobic

Kill obligately aerobic organisms including microorganisms, fish and invertebrate animals

Decomposition of these dead organisms
Wastewater Collection

http://www.winnipeg.ca/waterandwaste/sewage/systemOperation.stm
Wastewater Treatment: General Concepts

Stage I (Physical treatment)
- remove solid and reduce BOD

Stage II (Biological treatment)
- a smaller portion of the dissolved organic matter is mineralized; a large portion is converted from dissolved form to removable solids

Stage III (Chemical/physical/biological treatment)
- remove other organic components
Wastewater Treatment Plant
Primary (physical)
- Sewage
  - Comminute
  - Grit
    - Primary setting
      - Solid disposal
    - Trickling filter

Secondary (biological)
- Sludge digestor
  - Anaerobic tanks
  - Aeration tank
    - Secondary setting
      - Disinfection Chlorination
        - Effluent discharge
        - Dry disposal

Tertiary (chemical/Physical/biological)
- Chemical flocculation
  - Filter
    - Liquid
      - Solid disposal
Wastewater Treatment: General Concepts

BOD (Biological or biochemical oxygen demand)
- the amount of DO required by microorganisms to stabilize organic matter

COD (Chemical oxygen demand)
- the amount of oxygen required to oxidize organic matter

BOD < COD

Biological Treatment
- Rely on microbial activity
- Aerobic or anaerobic
- Associated with surface films or homogeneously suspended
Introduction

Most organic and inorganic chemicals are subject to enzymatic attack through the activities of living organisms

Type of pollutants

- **BTEX** (Benzene, Toluene, Ethylbenzene, Xylene)
- **PAH** (Polycyclic aromatic hydrocarbon)
- **Nitroaromatic compounds**
- **PCB** (Polychlorinated biphenyls)
- **Chlorinated aliphatic compounds**
- **Heavy metals** (Cd, Cu, Pb…)
- **Nuclear wastes**
- **Agricultural chemical wastes** (Triazine, DDT)
Fate of Pollutants

- Mineralized and converted to completely oxidized products, such as CO$_2$
- Transformed to another compound that may be toxic or nontoxic
- Accumulated within an organism
- Polymerized or bound to natural materials
Bioremediation

the productive use of biodegradative process to remove or detoxify pollutants that have found their way into the environment and threaten public health, usually as contaminants of soil, water and sediments

Definition

a pollution treatment technology that uses biological systems to catalyze the destruction or transformation of various chemicals to less harmful forms.
Bioremediation

INJECTION
Fluid Nutrients

RECOVERY

Carbon Dioxide

Oxygen and nutrients

Contaminant
Engineering of Bioremediation Process: Needs and Limitations

Consideration of selecting microbial process in bioremediation

**Characteristics of wastes:** Identify
- Appropriate microorganism or consortium
- Degradation pathway and rate
- Economically and functionally compete with incineration and chemical treatment
Engineering of Bioremediation Process:
Needs and Limitations

Process Analysis: Site characterization

The contaminants
- physical properties such as water solubility, octanol/water partition coefficient (ratio of comp. conc. in octanol phase to its aqueous phase of a two-phase system), vapor pressure (liquid-air partition), Henry’s Law constant \( (P_A = H_A X_A) \)

Their concentration
- sorption to soil particles and organic matters in soil determine the bioavailability.

The extent of contamination
- Distribution → Groundwater or soil treatment; Extent → Soil excavation and treatment.
Engineering of Bioremediation Process: Needs and Limitations

Process analysis: Microbial characterization
Biodegradation rate
- Population, lack of MO capable of degradation
Optimal conditions
- pH, temperature, DO

Process analysis: Environmental factors
Chemical analysis
- pH, COD, TOC, N, S, P, Fe…etc.
Physical analysis
- Soil type, clay content, org. matter content, particle size distribution
Microbial analysis
- BOD, cell counts
## Bioremediation: Reactor Option

**Groundwater remediation: ex situ**
- Activated sludge reactor
- Fixed film reactors

**Soil remediation**

<table>
<thead>
<tr>
<th>Method</th>
<th>Advantages</th>
<th>Disadvantages</th>
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<tbody>
<tr>
<td>Land farming</td>
<td>Simple and inexpensive</td>
<td>Long incubation time</td>
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<td></td>
<td>Currently accepted method for petroleum contaminants</td>
<td>Residual contamination</td>
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<td></td>
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<td>Require lining, monitoring and prevention of leakage</td>
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<tr>
<td>Slurry reactor</td>
<td>Good control of reactor condition</td>
<td>Expensive</td>
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<td></td>
<td>Aerobic or anaerobic</td>
<td>Limited by reactor size</td>
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<tr>
<td></td>
<td>Enhance desorption from soil</td>
<td>Require soil pretreatment</td>
</tr>
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<td></td>
<td>Short incubation time</td>
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Bioremediation in Soil

The inorganic solid phase in soil

- Inorganic solid components include crystalline materials in the form of layer silicates as well as more poorly crystalline oxides, hydroxides and oxyhydroxides (collectively termed hydrous oxides).

Natural organic materials in soils

- Recognizable compounds: large polymeric materials (cellulose, protein, lipid), simple molecules (sugars, organic acids, amino acids).

Physical characteristics of soil

- Soil by size: sand (2-0.05 mm), silt (0.5-0.002 mm), clay (<0.002 mm)
- c.a. 50% of a typical surface soil is pore space
Impact of contaminant bioavailability

- Bound substrates are not microbially available until desorption.

- The relatively strong interaction of cationic contaminants with negatively charged soil constituents decreases bioavailability

Transport phenomena

- Synthetic organic compounds + Mobile colloids (as carriers) → Increase rate on the soil

- EPS enhance the transport of organic contaminants

Abiotic catalysis

- Include: hydrolysis, substitution, redox, polymerization
Influence of Soil Properties on Microorganisms

Microbial survival

- Clay → Enhance microbial survival → Prevent from desiccation

Microbial mobility and transport

- In situ bioremediation: significant vertical transport of microorganisms in soil is necessary

Main processes governing bacterial transport:

- Sorption to soil components via hydrophobic interaction
- Physical filtering
- Inorganic or organic colloids are negatively charged → electrostatic repulsion
- Smaller-sized soil particles → less vertical transport
- Increase flow rate → increase cell transport
Biodegradation of Hydrocarbons

- Hydrocarbons of C_{10}-C_{22} are the least toxic and most biodegradable.
- Hydrocarbons of C_{5}-C_{9} have solvent-type toxicity at high concentrations.
- Highly condensed aromatic and cycloalkane systems, particularly those with four or more rings, are most resistant to biodegradation.
- Saturated, straight chain alkanes are most readily biodegradable.
- Double bond increases the resistance of degradation.
Planktonic versus Biofilm

Planktonic: Contamination–Infection Continuum

Biofilm Development

Attachment

Microcolony formation

Quorum sensing (signaling)

Mature biofilm

2006, B. Dyreke
Immune System

Host Defenses

Innate & nonspecific

Acquired & specific

Cells, tissues

Physical barriers

Chemical mediators

Cells, tissues

Memory

Discrimination, self/nonself

Granulocytes
Macrophages
Dendritic and NK cells

Skin mucous membranes

Defensins
Lysozyme
Complement

T Cells
B Cells

Opsonization

Resident responders

Inflammation
Cell cooperation
Immunity to infection

PPR: pattern-recognition receptors

Role of pathogen-derived molecules in promoting the induction of regulatory T cells versus $T_H^1$ and $T_H^2$ cells.
Human leukocytes actively phagocytose planktonic *S. aureus* but not *S. aureus* within the biofilm.

Establishment of *S. aureus* biofilms in vivo

S. aureus biofilms evade TLR2 and TLR9 recognition in vivo

Both TLR2 and TLR9 ligands are present within S. aureus biofilms (i.e., lipoproteins/PGN and eDNA, respectively),

Biofilms and Inflammation

Costerton and Stewart  Science Vol 284, 1999
S. aureus biofilms actively attenuate host proinflammatory responses
Macrophages actively phagocytize planktonic *S. aureus* but not biofilm-associated bacteria in vitro.

Macrophage invasion into biofilms in vitro is associated with cell death

Enhancing Phagocytosis

Engineering Infection Immunity