What is pollutant?

Very Frequent pollutants in environment

- Crude Oil
- Chlorinated organic
- Plastics
- Dyes

Maier et al., 2000: A Textbook of Environmental Microbiology, Academic Press, San Diego, California
**Crude Oil**

- **Major groups**
  - **Aliphatics**
    - Straight, branched & cyclic
    - \( \text{CH}_3(\text{CH}_2)_{16}\text{CH}_3 \)
  - **Aromatic**
    - Low- and High-molecular -- PAHs
    - Phenanthrene
    - Chrysene
    - Benzo(a)pyrene
  - **Resin**
  - **Asphaltenes**

- **Natural Oil Seeps**
  - 62% Consumption
  - 33% Natural Oil Seeps
  - 4% Transportation
  - 7% Exploitation

- **3 Billion ton per-year**

Source: Ilyas et al., 2011

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**Chlorinated organic**

- PCDDs
- PCDFs
- PCBs

- Coastal water and riverine

Source: Ilyas et al., 2011
10-11% to be world waste (Fukushima et al., 2011)

Synthetic Plastic

130 - 245 million ton per year (Formin, 2001 and Dacko et al., 2008)

Classification of the biodegradable polymer

Synthesis methods to obtain high molecular weight

Dyes/Colored substances

1000 dyes type – production 700000 ton/years

40% generated higher colored wastewater

Significant sources of water pollution

The use of dyes:

1. paper printing,
2. foods,
3. color photography,
4. textile dyeing,
5. pharmaceutical,
6. painting and,
7. other fields to support human daily-life.
**What is Bioremediation?**

Hamer, 1993 → “biological response to environmental abuse”, is the use of living organisms, primarily microorganisms, to degrade environmental pollutants into less toxic forms or non-toxic compounds.

Vidali, 2001 and Boopathy, 2001 → the usage of naturally occurring bacteria, fungi, or plants to purify hazardous materials.

Watanabe, 2001 → Utilization of the metabolic potential of organisms and the enzymes they produce by microorganisms.

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**Phenanthrene degradation**

 Diagram showing the degradation pathway of phenanthrene by microorganisms, including key intermediate compounds such as Phenanthrene, Phenanthrene 9,10-oxide, Phenanthrene 9,10-dihydrodiol, Phenanthrene 9,10-quinone, 2,2-Diphenic acid, 1,2-Benzedicarboxylic acid, Benzoic acid, and 4-hydroxybenzoic acid. The pathway also includes the prediction of phenanthrene degradative pathway by AS03.
**2,4,8-TCDF degradation**

<table>
<thead>
<tr>
<th>2,4,8 TCDF</th>
<th>Glucose</th>
<th>2,4,8 TCDF degradation (%)*</th>
<th>Enzyme activity ([U/L])**</th>
</tr>
</thead>
<tbody>
<tr>
<td>conc. (ppm)</td>
<td>b (d)</td>
<td>t (d)</td>
<td>r</td>
</tr>
<tr>
<td>0</td>
<td>0.01</td>
<td>4.97</td>
<td>0.97</td>
</tr>
<tr>
<td>1</td>
<td>0.04</td>
<td>3.92</td>
<td>0.99</td>
</tr>
<tr>
<td>10</td>
<td>0.08</td>
<td>3.27</td>
<td>0.96</td>
</tr>
</tbody>
</table>

* Values in parentheses are mycelium biomass of fungus (mg)

** Values in parentheses are incubation time (d) indicated the highest enzymes activities

**** nd = not detection

Lac = laccase

MnP = manganese peroxidase

Lip = lignin peroxidase

1,2-D = 1,2-dioxynase

2,3-D = 2,3-dioxynase

r = determination coefficient

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**HIDAYAT & TACHIBANA, 2013 : International biodeterioration & biodegradation**

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**Chemical structures and degradation pathways**

- 2,4,8-Trichloro-dibenzofuran
- 3,5-dichloro-2-hydroxybenzoic acid
- 3',5'-dichlorobiphenyl-2,2',5-triol
- 3,5,5'-trichlorobiphenyl-2,2'-diol
- (Z)-4-chloro-6-(3,5-dichlorophenyl)-2-hydroxy-6-oxohex-2-enoic acid
- 2,4,8-Trichloro-dibenzofuran (Z)-4-chloro-6-(3,5-dichlorophenyl)-2-hydroxy-6-oxohex-2-enoic acid
- 5-chloro-2-hydroxybenzoic acid
- 2,5-dihydroxybenzoic acid
- CO₂

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**Degradation and biomass weight**

- 2,4,8 TCDF degradation (%)
- Weight of mycelium biomass (mg)

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**Degradation and biomass weight**

- 2,4,8 TCDF degradation (%)
- Weight of mycelium biomass (mg)
The activity of manganese peroxidase was 0.01; 0.14; 0.20 and 0.26 U mg⁻¹ after 1, 2, 3 and 6 months incubation, respectively. Thus indicated that degradation of PLA/kenaf composite by *P. ostreatus* indeed occurred via oxidation reaction.

By the extension of incubation time, the circle hole became bigger and it was not appear again and kenaf fiber became shorter at the end incubation.

These indicated that circle hole might be the result of enzyme action to break down PLA on the surface and *P. ostreatus* also consumed kenaf fiber as energy and carbon sources.
Decolorization of waste water (type-2) by immobilized fungus U97. \( \text{MnP} = 1.41 \text{ U/L reaction} \)
\( \text{Lac} = 2.28 \text{ U/L reaction} \)

Flow rate = 1.5 mL/min

**Graphs:**
- Decolorization (%)
- Reaction time (h)

**Images:**
- Immobilized Fungus U97
- Kriks liquid medium, Autoclaving 121°C, 20 min (20 ml/flask)
- pH 4.5
- 1.5% Na-Alginate
- 0.1M CaCl₂
- Control 1 times 2 times 3 times
- Decolorization (%)
- Reaction time (h)

**Legend:**
- IM fungu U97
- IM enzyme U97

**Diagram:**
- Dyes degradation
- Incubation: 10 days
- Homogenized Fungus U97
- pH 7.12
- Solid cont = 0.29%

**Notes:**
- Fungus U97
Degradation pathway of an anthraquinone dye, Reactive Blue 5 catalyzed by a dye-decolorizing peroxidase, DyP from *Thanatephorus cucumeris*. Dec 1. Electrospray ionization mass spectrometry (ESI-MS), TLC and 1H- and 13C-NMR. Sugano et al., 2009.

Fluidised-bed bioreactor (FBBR)

Continuous-stirred tank reactors (CSTRs)

Enzymes
- Green Color
- Rotten egg odor
- Water deep, 2.6-3 M
- Sediment deep, 0.6-0.8 M
- pH 7-8

Why?

- **Green Color, Dyes/Color substrates?**

From Where?

Water Input
Occur in stagnant water (Fresh or sea Water)

Chlorophyll is not soluble in water & soluble in alcohol, methanol, AtoAC.
Algae tend to grow very quickly under high nutrient availability, but each alga is short-lived, and the result is a high concentration of dead organic matter which starts to decay with hypoxic condition.

Cause algae grow

1. Sunlight

Swim toward the light source

Wild type

Control +ROS +ROS quencher

agg1

Treatment, keep 10 days.

Before After
2. Nutrients, ammonia - NH₃, nitrite – NO₂⁻, nitrate – NO₃⁻, phosphate – PO₄³⁻
   Fish waste, decaying leaves, uneaten food, household waste, minerals from tap water etc. provide food for excessive algae growth

3. Warm Water
   Often caused by shallow ponds, inadequate plant cover, poor circulation

Algae Blooms in MWB Lake

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Microbial

> 3.1 X 10⁹
  Fungi : > 2.8 X 10⁹ CFU/mL
  Non Fungi : > 3.7 X 10⁸ CFU/mL

< 1 x 10⁵ CFU/mL

Disease-causing bacteria that can be transmitted by water include Vibrio cholerae, Salmonella sp, Campylobacter sp, Shigella sp, and Staphylococcus aureus. Diseases: cholera, gastroenteritis, typhoid fever, dysentery, diarrheas.
**Rotten egg odor**

Decaying organic deposits in underground

H$_2$S (Hydrogen sulfide) = rotten egg odor

Caused corrosive, tarnish silver rapidly and toxic to

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**Water Treatments Test**

- Carbon, 3 days 50 g/1 L
  - Before
  - After

- F$_2$O$_3$, 3 days 3%
  - Before
  - After

- Sunlight
  - Before
  - After

- Alga Killer, 300 ppm = Fail =
  - Before
  - After

- Fungi, Bacteria ?!

- Quartz, ordering
- First Aid, ordering

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1. Alga Killer, 300 ppm

2. Quartz, ordering

3. First Aid, ordering
1. Make sure water input output well.

2. Try to obtain a natural biological balance. (Combination of Clean Water Plant material (oxygenating, filter and algae competitor) will result in clean water without chemical treatments)
3. Keep surface water shading 50-60% with floating plants.

4. Keep good water aeration and circulation.

5. Added an ultraviolet sterilizer combined with aeration system to kill algae.

6. Keep the number fish reasonable level.

7. Make sure a good maintenance and handling.
Thanks You