Intelligent PCBs
Detector and Degrader

Beijing Normal University iGem Team
School of Environment
Outline

♠ Background
♠ Problems & Our solutions
♠ Tentative experiments
♠ Difficulties
♠ Our accomplishments
♠ Future studies
♠ Acknowledgements
The “Big Brother”

Polychlorinated biphenyls (PCBs): a class of organic compounds with 1 to 10 chlorine atoms attached to biphenyl, composed of two benzene rings, by replacing the hydrogen atoms.

They are tough: low water-solubility, low vapor pressure, high dielectric constants, very high thermal conductivity, extremely resistant to oxidation, reduction and elimination.

\[ \text{C}_{12}\text{H}_{10-x}\text{Cl}_x \]
“The big brother is watching you”

- PCBs were widely used in electrical equipments, such as transformers, capacitors, since they are tough.
- But, they are extremely toxic and difficult to fully degrade. They can accumulate in animal bodies, such as the fat of fishes.
- PCBs were manufactured in the United States from 1929 until their manufacture was banned in 1979.
- They caused a lot of environmental incidents worldwide, including the problem in Hudson River and Great Lakes in US.
The silent Hudson River

- GE released up to 1,300,000 pounds (590,000 kg) of PCBs into the Hudson River between approximately 1947 and 1977.
- In 1984, attempts to cleanup the Upper Hudson River began, including the removal of 180,000 cubic yards (140,000 cubic meters) of contaminated river sediments near Fort Edward.
- In 2002, the United States Environmental Protection Agency announced to remove 2,650,000 million cubic yards (2,030,000 cubic meters) contaminated sediments in the Upper Hudson River.
- We still cannot eat the fishes in Upper Hudson River until now.
Sphingomonas was defined in 1990 as a group of Gram-negative, rod-shaped, chemoheterotrophic, strictly aerobic bacteria. They have been used to degrade many polymers. One strain, Sphingomonas sp. 2MPII, can degrade 2-methylphenanthrene.  
Sphingomonas can degrade over 40% of the weight of plastic bags (Polyethylene) in less than 3 months.  
They can also work out PCBs.

References:
Enzymes Responsible for Oxidative Degradation of PCBs

- PCB degradation is a cometabolism by four enzymes.
- Biphenyl Dioxygenase (BphA)
- Dihydrodiol Dehydrogenase (BphB)
- 2,3-Dihydroxybiphenyl Dioxygenase (BphC)
- Hydrolase (BphD)
Enzymes Responsible for Oxidative Degradation of PCBs

FIG. 1. Catabolic pathway for degradation of biphenyl and organization of bph gene cluster in *Pseudomonas pseudoalcaligenes* KF707. Compounds: I, biphenyl; II, 2,3-dihydroxy-4-phenylhexa-4,6-diene (dihydrodiol compound); III, 2,3-dihydroxybiphenyl; IV, 2-hydroxy-6-oxo-6-phenylhexa-2,4-dienoic acid (biphenyl meta-cleavage compound: HOPDA); V, benzoic acid; VI, 2-hydroxypenta-2,4-dienoic acid. Enzymes: BphA1-BphA2-BphA3-BphA4, biphenyl dioxygenase; BphB, dihydrodiol dehydrogenase; BphC, 2,3-dihydroxybiphenyl dioxygenase; BphX0, glutathione S-transferase; BphX1, 2-hydroxyxypenta-2,4-dienoate hydratase; BphX2, acetaldehyde dehydrogenase (acylating); BphX3, 4-hydroxy-2-oxovalerate aldolase; BphD, 2-hydroxy-6-oxo-6-phenylhexa-2,4-dienoic acid hydrolase. The BphR1 protein, belonging to the GntR family, is a transcriptional regulator involved in the expression of bphR1 and bphX0/X1/X2/X3/D. The function of orf3 remains unclear (from Refs. 163 and 179).

Source: Kensuke Furukawa and Hidehiko Fujihara, MicrobialDegradation of Polychlorinated Biphenyls: Biochemical and Molecular Features, Journal of bioscience and bioengineering, 105:433–449 (2008), permitted to use by Kensuke Furukawa
System Design

- Initiating the system
- Handling reaction bottleneck
- Enhancing degradation efficiency by controlling the solubility of PCBs
- Amplifying output signal
Initiate the System

Awakening the system by the presence of PCBs.
Promoter: *PbphR1*
Regulator: *bphR2*

The bottleneck

- BphA catalyzes the initial 2,3-dioxygenation to obtain dihydrodiol compound.
- BphB catalyzes the conversion of dihydrodiol to dihydroxy compound.
- But, dihydroxy compound is lethal to the bacteria and suppresses the activity of BphC.
- Our solution: suppress the activity of BphA and BphB to reduce the production of dihydroxy compound.

Solving the Bottleneck

sRNA system: sodB & rhyB

*rhlAB* adds the pathway to produce biosurfactant which increases the solubility of PCBs, therefore, more PCBs can enter the cell.
Increase Sensitivity

We add T7 system to amplify the output signal. Therefore low concentration of PCBs can be detected through this amplifier.

Consider the model:

\[ A + B \ (k1) \rightarrow C + F \]
\[ A + C \ (k2) \rightarrow D + F \]
\[ A + D \ (k3) \rightarrow E + F \]

The derivatives can be written as:

\[ \frac{dA}{dt} = -k_1 AB - k_2 AC - k_3 AD \]
\[ \frac{dB}{dt} = -k_1 AB \]
\[ \frac{dC}{dt} = k_1 AB - k_2 AC \]
\[ \frac{dD}{dt} = k_2 AC - k_3 AD \]
\[ \frac{dE}{dt} = k_3 AD \]

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Why MCMC

- If we have already known the parameters:
  - Use numerical methods to solve ODE system:
    - Euler Method
    - 4th Order Runge-Kutta Method
    - Quasi-Newton Method

However, what if we **don’t** know parameters but observation data?
- **Bayesian Inference** given the observation, how to make inference on parameters?

\[
P(H|E) = \frac{P(E|H)P(H)}{P(E)}
\]

\[
\text{posterior} = \frac{\text{likelihood} \times \text{prior}}{\alpha}
\]

**Monte Carlo** a class of computational algorithms that rely on repeated random sampling to compute their results. Often used when simulating mathematical systems (e.g. numerical integration).

**Markov Chain** stochastic process with Markovian Property (future is only related to present, independent to past). Some Markov Chains have stationary distribution which is very useful for MCMC.
Markov chain Monte Carlo (MCMC) is the idea of using simulations $X_1, \ldots, X_n$ of a Markov chain to approximate expectations

$$\mu = E_\pi\{g(X_i)\}$$

by sample averages

$$\hat{\mu}_n = \frac{1}{n} \sum_{i=1}^{n} g(X_i)$$

where $\pi$ is the equilibrium distribution, also called invariant distribution, stationary distribution, or ergodic limit of the Markov chain (assuming such exists).
4 Simple Steps of MCMC

1 **Specify** the model
   - (parameter priors, likelihood function, initial values).

2 **Generate** a Markov Chain whose stationary distribution is the desired density.

3 **Sample** from posterior distribution.

4 **Infer** from posterior distribution (e.g. Mean, STD, MC error, etc).

![Graph showing prior, likelihood, and posterior distributions](image)
MCMC Algorithms

1 Metropolis-Hastings Sampler
- Generates a random walk using a proposal density and a method for rejecting some proposed moves.

2 Gibbs Sampler
- Special case of Metropolis-Hastings sampler, samples from full conditional distribution and thus does not reject proposed moves. My favorite!

Reference Sites:
- [http://www.mrc-bsu.cam.ac.uk/bugs/](http://www.mrc-bsu.cam.ac.uk/bugs/)
- [http://www.helsinki.fi/~mjlaine/mcmc/examples.html](http://www.helsinki.fi/~mjlaine/mcmc/examples.html)
Tentative Experiments

Template: 2,4,5-PCBs
We test the function of promoter PpcbC.

Results: The promoter responds insensitive to high chlorined PCBs.
Our Accomplishments

♠ Seven parts in Biobrick format
   \textit{bphB bphC bphD dxnA dxnB dbfB redA2}

Standard vector \textit{pSB1AC3}

♠ Experiments protocols
   Experimental details provided on our Wiki.

♠ Special protocols
   Modified Hot start PCR;
   Several Effective additives

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