Final Report

Decay of Toluene in Sol-Gel Encapsulated *Pseudomonas putida*

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Background

Toluene is a solvent found in gasoline and toluene exposure has been linked to central nervous system dysfunction (ATSDR, 2000) for both short-term and long-term exposure. *Pseudomonas putida* is a gram-negative bacterium that naturally breaks down toluene and incorporates the products into its metabolic cycle.

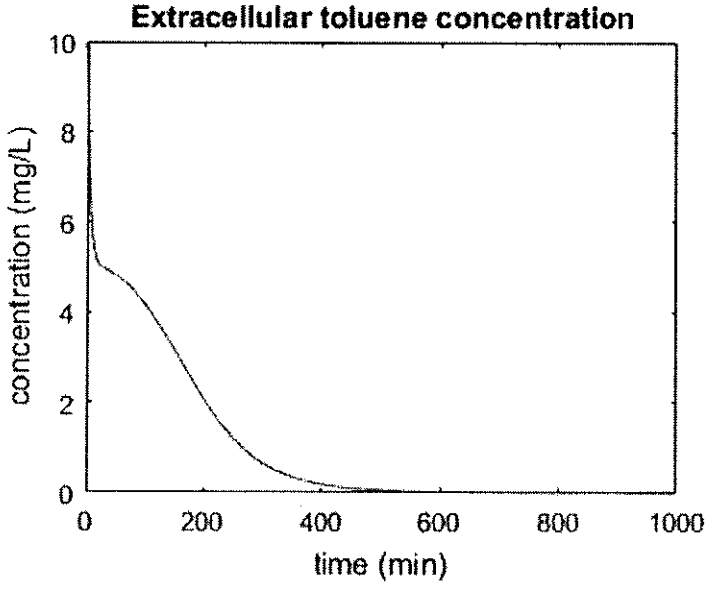
Once transported into *P. putida* by membrane protein TodX, toluene catalyzes the phosphorylation of TodS, the first protein in a two-component regulatory pathway (Wang et al., 2994). TodS then transphosphorylates TodT, which serves as an activator of the PTodX promoter (Lacal et al., 2006). Up to five phosphorylated TodT proteins bind to the promoter, causing increasing levels of activation of the gene (Lacal et al., 2008). The PTodX promoter controls transcription of the Tod cassette, which houses both the TodX membrane transport protein and the enzymes responsible for the degradation and incorporation of toluene into the citric acid cycle. The first enzyme in this pathway is toluene dioxygenase.

To act as a biosensor and bioreporter, *P. putida* TVA8 was engineered by inserting a lux gene cassette behind a copy of the PtodX reporter (Applegate et al., 1998). The assembly was then inserted via mini-transposon delivery. Thus, when the PtodX promoter is active, luciferase, a fluorescent protein, is expressed along with the enzymes of the toluene degradation pathway. A shortcoming of biosensors is that their lifetime is contingent upon the organisms remaining alive. TVA8 cells have been encapsulated in various formulations to determine the method that maintains functionality while extending cell viability. It has been determined that a TMOS alcohol-free sol-gel encapsulation is the formulation that best serves this goal (Zhang, 2008).

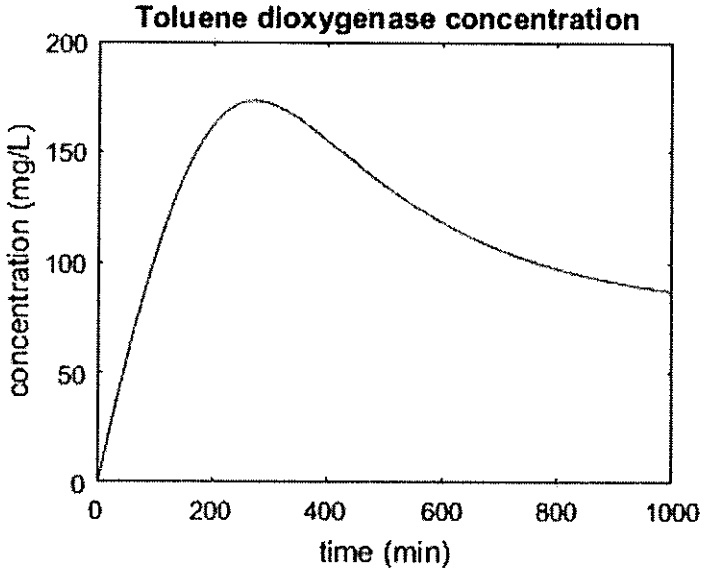
Model Derivation

The first mass component of the system is the toluene. It was assumed that the toluene solution was induced instantaneously and uniformly throughout the sol-gel slab. To account for the diffusion of the toluene from the sol-gel matrix into the cells, a pooled model was used. One variable, Tout (units of mg/L), represented the concentration of toluene outside of the cells. Another variable, Tin (also units of mg/L), represented the concentration of toluene inside the cells. As toluene requires the TodX transport protein to move into the cell, the rate of transport into the cells was defined as the difference in concentrations multiplied by the concentration of TodX. As this system is closed and contains a finite amount of toluene, the accumulation of Tout was set equal to the negative of this rate (Equation 1). The constant of proportionality k5 was parametrized and set to 0.1 for the final model.

$$\frac{dT_{out}}{dt} = -k_5(T_{out} - T_{in})Todx \qquad (1)$$

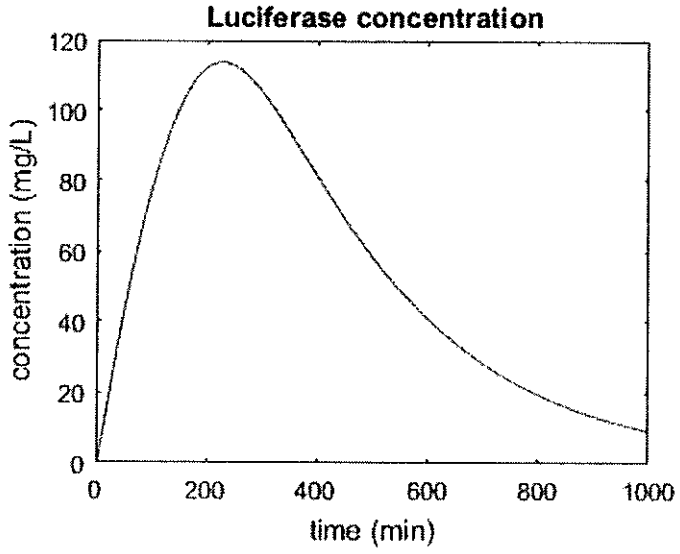


The generation of TodX (in mg/L) was modeled using a hill function and it was assumed that TodX decays at a constant rate (Equation 2). The constants of the hill function were assumed to

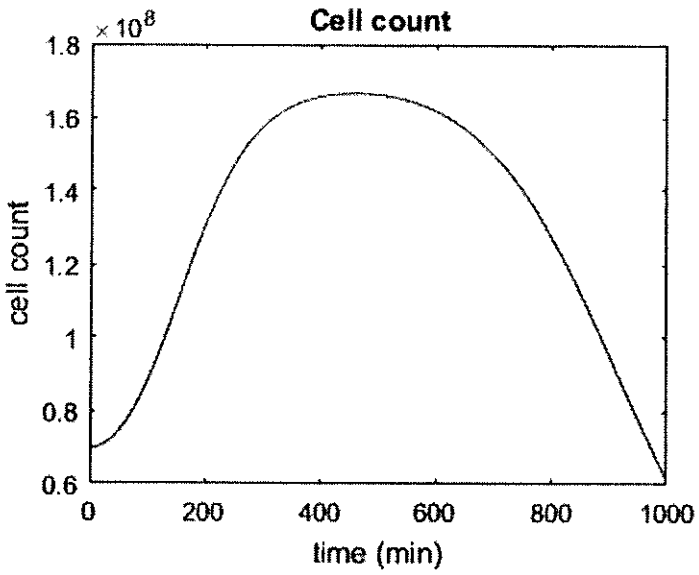


Similarly, luciferase , L (mg/L), is expressed under the Ptodx promoter so it was assumed to follow the same generation kinetics. The half-life of luciferase was found to be 180 minutes and so the decay rate was set to log(2)/180 (Equation 4).

$$\frac{dL}{dt} = \frac{V_{m,lucif}T}{K_{m,lufic}+T} - k_1 L \qquad (4)$$



Toluene is consumed by the toluene dioxygenase enzyme so its consumption rate was set proportional to the concentration of enzyme and the concentration of itself, the substrate. This


Cell count
× 10^8
1.8
1.6
1.4
1.2
1
0.8
0.6
cell count
0
200
400
600
800
1000
time (min)

Nomenclature Summary

Variables

Parameter Symbol	*Meaning*	*Units*
T_{in}	concentration of toluene inside the cells	[mg/L]
T_{out}	concentration of toluene outside of the cells	[mg/L]
C	number of cells	[cell number]
L	concentration of luciferase	[mg/L]
E	concentration of toluene dioxygenase	[mg/L]
Todx	concentration of todx promoter	[mg/L]

Constants

Symbol	*Meaning*	*Value*	*Source*
k_3	growth constant	10^{-5}	parametrized
k_4	death constant	10^{-5}	parametrized
klucif	max luciferase transcription rate	2	Kelly et al. 2000
Klucif	half-saturation luciferase transcription rate	5	Kelly et al. 2000
$T_{1/2}$	luciferase half life	180	Thompson et al. 1991
k_5	toluene degradation constant	0.1	parametrized
ktol	max tod transcription rate	2	Kelly et al. 2000
Ktol	half-saturation tod transcription rate	5	Kelly et al. 2000
$T_{1/2,Todx}$	Todx half life	50	parametrized

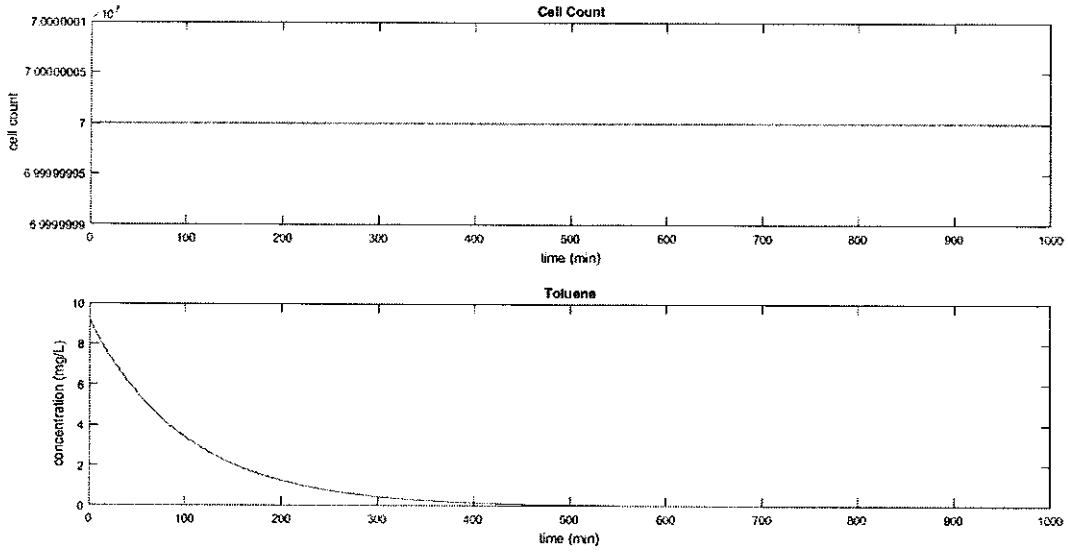
Appendix A

This section contains the history of iterations that led to the development of this model. Each iteration is described with a diagram of the system, a list of the variables modeled, the core assumptions that are modeled, the equations that were used to model the variables, the initial conditions, the parameter values, and graphs that represent the output of that iteration of the model.

For sake of simplicity, the iterations have been summarized in Table 1 and been given page numbers for reference.

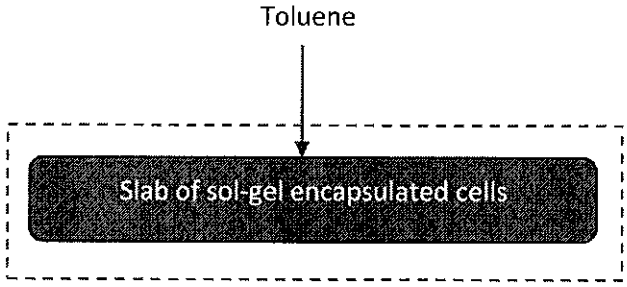
Iteration	*Significant Changes*	*Page Number*
0	base model	14
0.1	nonconstant toluene degradation	17
0.2	adds luciferase concentration variable	20
0.3	adds luciferase decay term	23
1	adds hill function for generation of luciferase	26
2	cell growth rate dependent on toluene consumption rate	30
3	adds cell death term	34
4	cell death inversely proportional to toluene	38
5	adds toluene dioxygenase variable	42
6	toluene degradation proportional to toluene dioxygenase	46
7	adds T_{out} variable	50
8	models toluene diffusion with Fickian diffusion	55
9 (final)	adds TodX variable	60

Model Output



Iteration 0.1

Picture



Parameters

Parameter Symbol	Meaning	Units
T	concentration of toluene	[mg/L]
C	number of cells	[cell number]

Assumptions

1. Instantaneous, homogenous distribution of toluene throughout the system
2. No cell growth or death
3. Degradation of toluene is proportional to amount of toluene **and cell count**

Equations

$$\frac{dT}{dt} = -k_1 TC$$

$$\frac{dC}{dt} = 0$$

Initial Conditions

T(0) = 9.2 mg/L
C(0) = $7*10^8$ cells

Parameter Values
K1 = 1E-10 (parametrized value)

Main Code

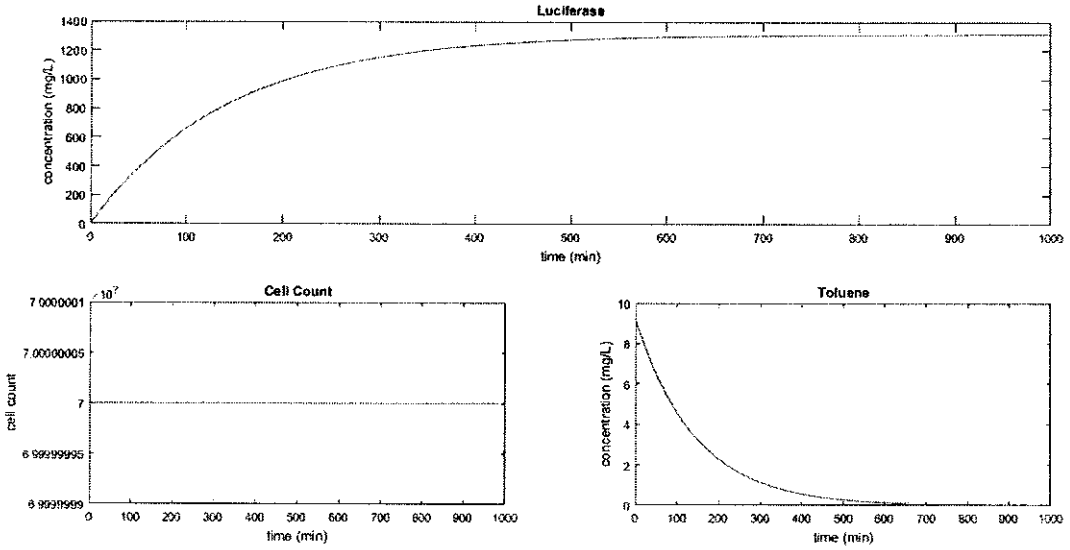
```
clc
clear

Time = [0 1000];
Toluene = 9.2; % initial Toluene concentration [mg/L]

[T, Y] = ode45('LuciferaseModelFunctions01', Time, [70000000, 9.2]);

subplot(2,1,1);
plot(T,Y(:,1),'r');
title('Cell Count');
xlabel('time (min)');
ylabel('cell count');
subplot(2,1,2);
plot(T,Y(:,2));
hold on
title('Toluene');
xlabel('time (min)');
ylabel('concentration (mg/L)');
```

Function Code

```
function A = LuciferaseModelFunctions01(~,Y)
A = zeros(2,1);

C = Y(1);
T = Y(2);

% Cell count is constant
A(1) = 0;

% Toluene level
k2 = 1E-10;
A(2) = -k2*T*C;

end
```

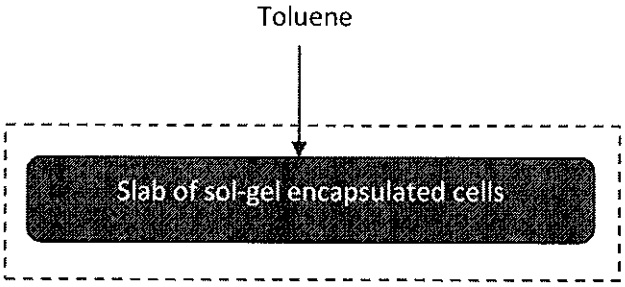
Parameter Values
K1 = 1
K2 = 1E-10 (parametrized value)

Model Output



Iteration 0.3

Picture



Parameters

Parameter Symbol	Meaning	Units
T	concentration of toluene	[mg/L]
C	number of cells	[cell number]
L	concentration of luciferase per cell	[mg/L]

Assumptions

1. Instantaneous, homogenous distribution of toluene throughout the system
2. No cell growth or death
3. Degradation of toluene is proportional to amount of toluene and cell count and **luciferase count**
4. Luciferase generation rate is proportional to toluene concentration
5. **Luciferase degradation is proportional to the luciferase concentration and is equal to log(2) divided by the half life of the protein**

Equations

$$\frac{dT}{dt} = -k_1 TCL$$

$$\frac{dC}{dt} = 0$$

$$\frac{dL}{dt} = k_2 T - \frac{\log(2)}{T_{1/2}} L$$

Initial Conditions

T(0) = 9.2 mg/L
C(0) = $7*10^8$ cells

Main Code

```
clc
clear

Time = [0 1000];
Toluene = 9.2; % initial Toluene concentration [mg/L]

[T, Y] = ode45('LuciferaseModelFunctions03', Time, [0, 70000000, 9.2]);

subplot(2,2,[1 2]);
plot(T,Y(:,1));
title('Luciferase');
xlabel('time (min)');
ylabel('concentration (mg/L)');
subplot(2,2,3);
plot(T,Y(:,2));
hold on
title('Cell Count');
xlabel('time (min)');
ylabel('cell count');
subplot(2,2,4);
plot(T,Y(:,3));
hold on
title('Toluene');
xlabel('time (min)');
ylabel('concentration (mg/L)');
```

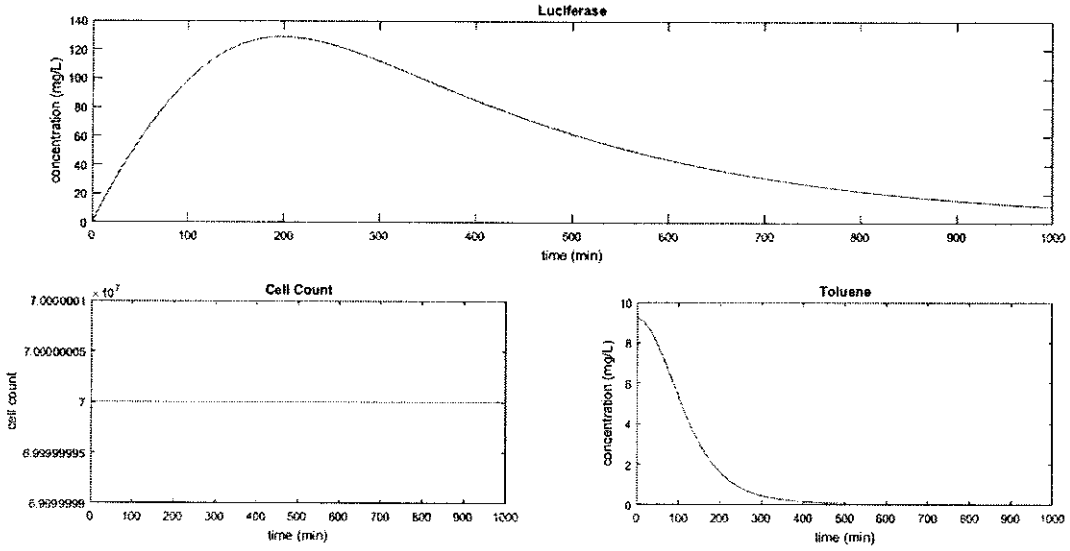
Function Code

```
function A = LuciferaseModelFunctions03(~,Y)
A = zeros(3,1);

L = Y(1);
C = Y(2);
T = Y(3);

% Luciferase generation is proportional to Toluene concentration
k1 = 1E-3;
HalfLife = 180;
AlphaL = log(2)/HalfLife;
A(1) = k1*T - AlphaL*L;

% Cell count is constant
A(2) = 0;

% Toluene level
k2 = 1E-10;
A(3) = -k2*T*C*L;

end
```

L(0) = 0 mg/L

Parameter Values
Vm = 2 (researched value)
Km = 5 mg toluene/L (researched value)
K1 = log(2)/toluene half life = .00167
K2 = 1E-10 – 1E-12 (parametrized value)

Model Output



Function Code

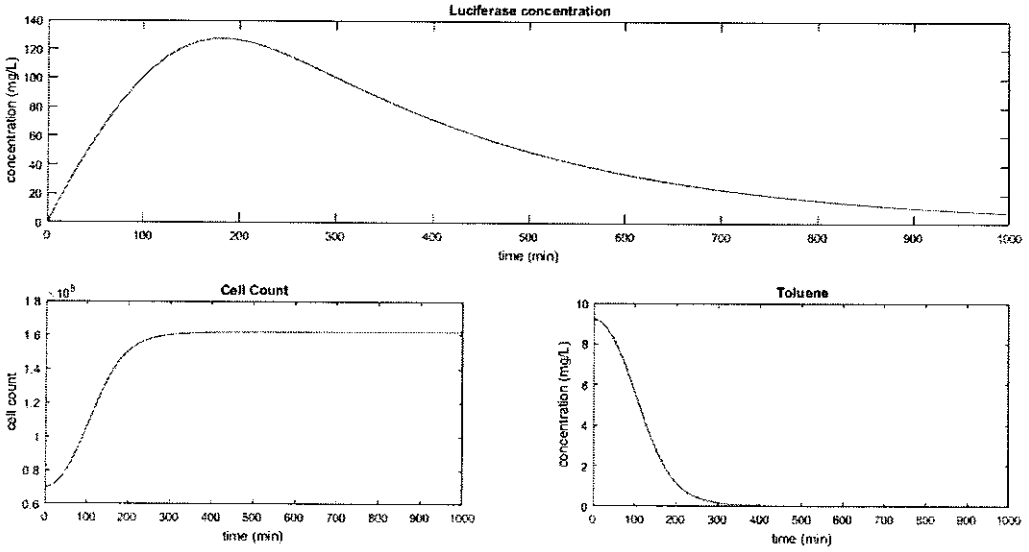
```
function A = LuciferaseModelFunctions1(~,Y)
A = zeros(3,1);

L = Y(1);
N = Y(2);
T = Y(3);

% Luciferase level
klucif = 2;    % [unitless]
Klucif = 5;        % [mg/L]

% calculate degradation rate
HalfLife = 180;      % [minutes]
AlphaL = log(2)/HalfLife;

A(1) = klucif*T/(Klucif+T)-AlphaL*L;

% Cell count is constant
A(2) = 0;

% Toluene level
k2 = .0001;
A(3) = -k2*L*T;

end
```

Initial Conditions

T(0) = 9.2 mg/L
C(0) = $7*10^8$ cells
L(0) = 0 mg/L

Parameter Values
Vm = 2 (researched value)
Km = 5 mg toluene/L (researched value)
K1 = log(2)/toluene dioxygenase half life = .00167
K2 = 1E-10 – 1E-12 (parametrized value)
K2 was set to 1E-12 for the output below
K3 = 1E-4 – 1E-6 (parametrized value)
K3 was set to 1E-5 for the output below

Model Output



Function Code

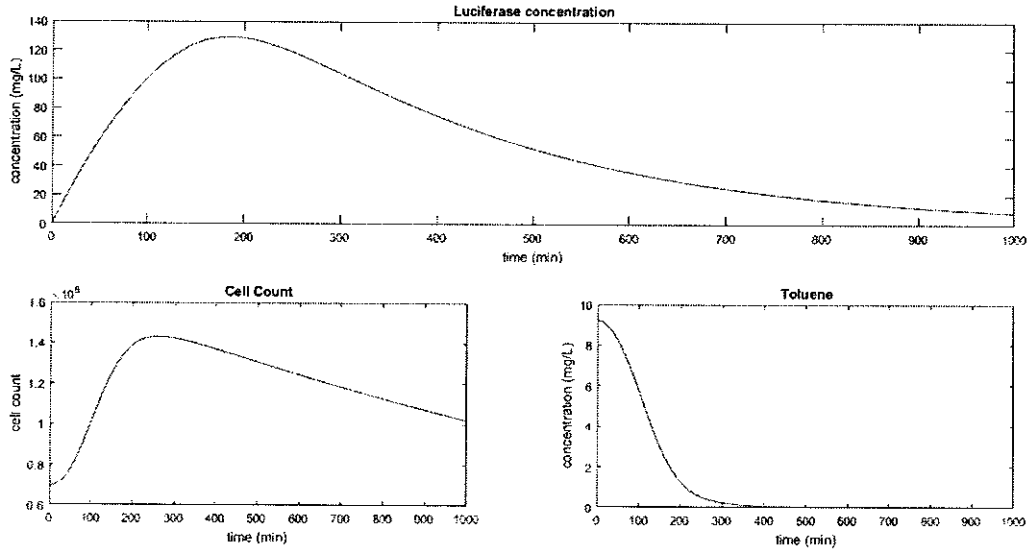
```
function A = LuciferaseModelFunctions2(~,Y)
A = zeros(3,1);

L = Y(1);
N = Y(2);
T = Y(3);

% Luciferase level
klucif = 2;    % [unitless]
Klucif = 5;        % [mg/L]

% calculate degradation rate
HalfLife = 180;      % [minutes]
AlphaL = log(2)/HalfLife;

A(1) = klucif*T/(Klucif+T)-AlphaL*L;

% Cells grow proportionally to toluene concentration
k3 = 1E-5;
A(2) = k3*N*L*T;

% Toluene level
k2 = 1E-12;
A(3) = -k2*N*L*T;

end
```

Initial Conditions

T(0) = 9.2 mg/L
C(0) = $7*10^8$ cells
L(0) = 0 mg/L

Parameter Values
Vm = 2 (researched value)
Km = 5 mg toluene/L (researched value)
K1 = log(2)/toluene dioxygenase half life = .00167
K2 = 1E-10 – 1E-12 (parametrized value)
K2 was set to 1E-12 for the output below
K3 = 1E-4 – 1E-6 (parametrized value)
K3 was set to 1E-5 for the output below
K4 = 1E-3-1E-5 (parametrized)
K4 was set to 5E-4 for the output below

Model Output



Function Code

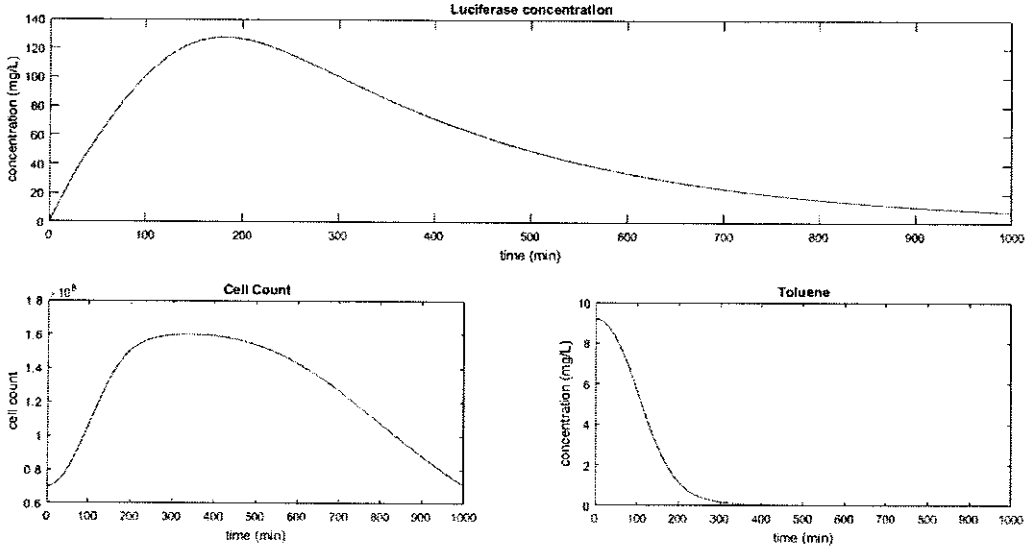
```
function A = LuciferaseModelFunctions3(~,Y)
A = zeros(3,1);

L = Y(1);
C = Y(2);
T = Y(3);

% Luciferase level
klucif = 2;    % [unitless]
Klucif = 5;        % [mg/L]

% calculate degradation rate
HalfLife = 180;      % [minutes]
AlphaL = log(2)/HalfLife;

A(1) = klucif*T/(Klucif+T)-AlphaL*L;

% Cells grow proportionally to toluene concentration
k3 = 1E-5;
k4 = 5E-4;
A(2) = k3*C*L*T-k4*C;

% Toluene level
k2 = 1E-12;
A(3) = -k2*C*L*T;

end
```

Initial Conditions

T(0) = 9.2 mg/L
C(0) = $7*10^8$ cells
L(0) = 0 mg/L

Parameter Values
Vm = 2 (researched value)
Km = 5 mg toluene/L (researched value)
K1 = log(2)/toluene dioxygenase half life = .00167
K2 = 1E-10 – 1E-12 (parametrized value)
K2 was set to 1E-12 for the output below
K3 = 1E-4 – 1E-6 (parametrized value)
K3 was set to 1E-5 for the output below
K4 = 1E-3-1E-5 (parametrized)
K4 was set to 1E-5 for the output below

Model Output



Function Code

```
function A = LuciferaseModelFunctions4(~,Y)
A = zeros(3,1);

L = Y(1);
C = Y(2);
T = Y(3);

% Luciferase level
klucif = 2;    % [unitless]
Klucif = 5;        % [mg/L]

% calculate degradation rate
HalfLife = 180;      % [minutes]
AlphaL = log(2)/HalfLife;

A(1) = klucif*T/(Klucif+T)-AlphaL*L;

% Cells grow proportionally to toluene concentration
k3 = 1E-5;
k4 = 1E-5;
A(2) = k3*C*L*T-k4*C/T;

% Toluene level
k2 = 1E-12;
A(3) = -k2*C*L*T;

end
```

$$\frac{dL}{dt} = \frac{V_m T}{K_m + T} - k_1 L$$

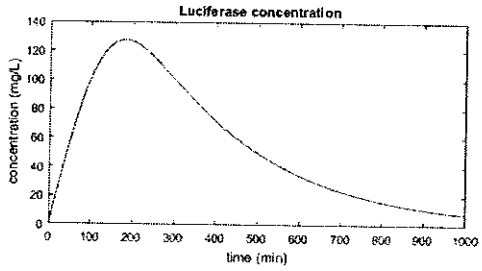
$$\frac{dE}{dt} = \frac{V_m T}{K_m + T} - k_1 L$$

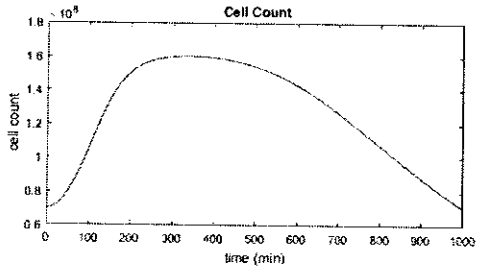
Initial Conditions

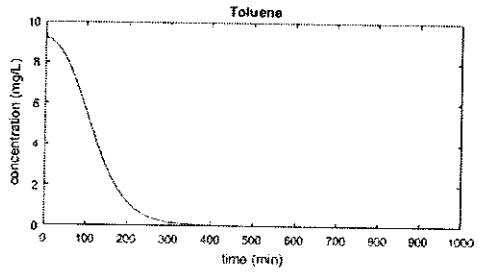
T(0) = 9.2 mg/L
C(0) = $7*10^8$ cells
L(0) = 0 mg/L
E(0) = 0 mg/L

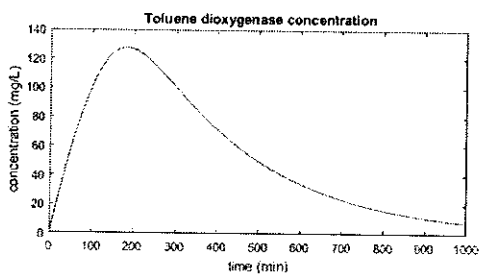
Parameter Values
Vm = 2 (researched value)
Km = 5 mg toluene/L (researched value)
K1 = log(2)/toluene dioxygenase half life = .00167
K2 = 1E-10 – 1E-12 (parametrized value)
K2 was set to 1E-12 for the output below
K3 = 1E-4 – 1E-6 (parametrized value)
K3 was set to 1E-5 for the output below
K4 = 1E-3-1E-5 (parametrized)
K4 was set to 1E-5 for the output below

Model Output









Function Code

```
function A = LuciferaseModelFunctions5(~,Y)
A = zeros(4,1);

L = Y(1);
C = Y(2);
T = Y(3);
E = Y(4);

% Luciferase level
klucif = 2;    % [unitless]
Klucif = 5;        % [mg/L]

% calculate degradation rate
HalfLife = 180;      % [minutes]
AlphaL = log(2)/HalfLife;

A(1) = klucif*T/(Klucif+T)-AlphaL*L;

% Cells grow proportionally to toluene concentration
k3 = 1E-5;
k4 = 1E-5;
A(2) = k3*C*L*T-k4*C/T;

% Toluene level
k2 = 1E-12;
A(3) = -k2*C*L*T;

% Toluene diozygenase level
A(4) = klucif*T/(Klucif+T) - AlphaL*L;

end
```

$$\frac{dL}{dt} = \frac{V_{m,lucif}T}{K_{m,lufic} + T} - k_1 L$$

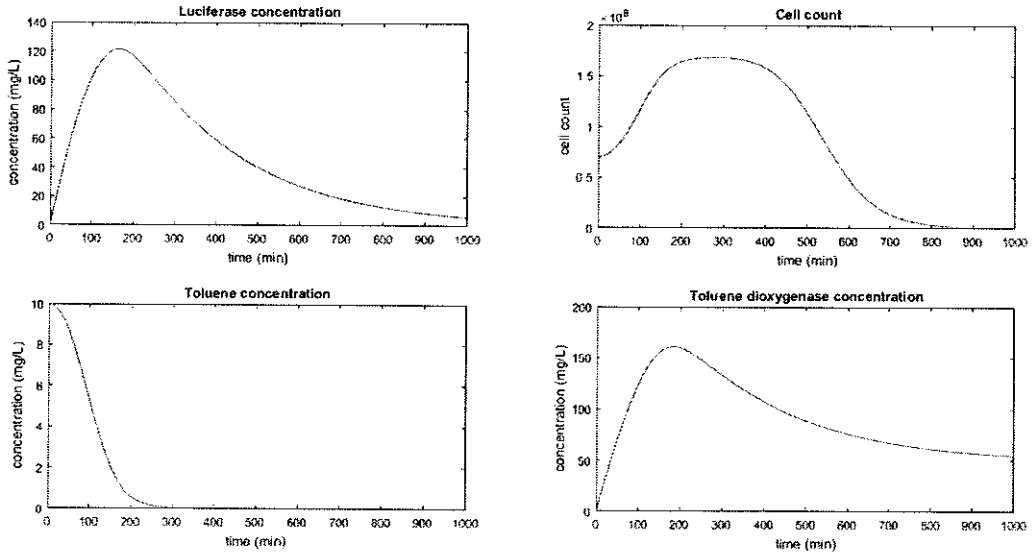
$$\frac{dE}{dt} = \frac{V_{m,tol}T}{K_{m,tol} + T} - k_1 \frac{dC}{dt} L$$

Initial Conditions

T(0) = 9.2 mg/L
C(0) = $7*10^8$ cells
L(0) = 0 mg/L
E(0) = 0 mg/L

Parameter Values
Vm,lucif = 2 (researched value)
Km,lucif = 5 mg toluene/L (researched value)
Vm,tol = 2
Km,tol = 5
K1 = log(2)/toluene dioxygenase half life = .00167
K2 = 1E-10 – 1E-12 (parametrized value)
K2 was set to 1E-12 for the output below
K3 = 1E-4 – 1E-6 (parametrized value)
K3 was set to 1E-5 for the output below
K4 = 1E-3-1E-5 (parametrized)
K4 was set to 1E-5 for the output below

Model Output



Function Code

```
function A = LuciferaseModelFunctions6(~,Y)
A = zeros(4,1);

L = Y(1);
C = Y(2);
T = Y(3);
E = Y(4);

% Cells grow proportionally to toluene concentration
k3 = 1E-5;
k4 = 1E-5;
A(2) = k3*C*E*T-k4*C/T;

% Luciferase level
klucif = 2;    % [unitless]
Klucif = 5;        % [mg/L]

% calculate degradation rate
HalfLife = 180;      % [minutes]
AlphaL = log(2)/HalfLife;

A(1) = klucif*T/(Klucif+T)-AlphaL*L;

% Toluene level
k2 = 1E-12;
A(3) = -k2*C*E*T;

% Toluene dioxygenase level
ktol = 2;
Ktol = 3;
A(4) = ktol*T/(Ktol+T) - AlphaL*L;

end
```

$$\frac{dT_{out}}{dt} = -k_5 T_{out}$$

$$\frac{dT_{in}}{dt} = k_5 T_{out} - k_2 CET$$

$$\frac{dC}{dt} = k_3 CET - k_4 \frac{C}{T}$$

$$\frac{dL}{dt} = \frac{V_{m,lucif} T}{K_{m,lufic} + T} - k_1 L$$

$$\frac{dE}{dt} = \frac{V_{m,tol} T}{K_{m,tol} + T} - k_1 L$$

Initial Conditions

Tout(0) = 9.2 mg/L
C(0) = $7*10^8$ cells
L(0) = 0 mg/L
E(0) = 0 mg/L
Tin(0) = .001 mg/L

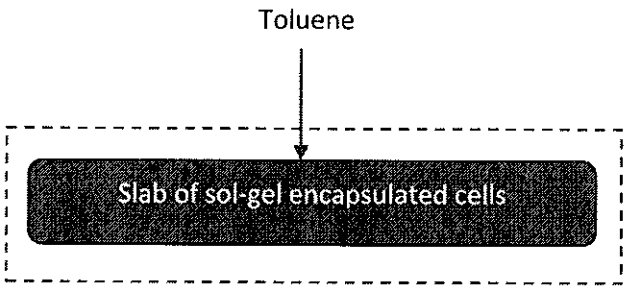
Parameter Values
Vm,lucif = 2 (researched value)
Km,lucif = 5 mg toluene/L (researched value)
Vm,tol = 2
Km,tol = 5
K1 = log(2)/toluene dioxygenase half life = .00167
K2 = 1E-10 – 1E-12 (parametrized value)
K2 was set to 1E-12 for the output below
K3 = 1E-4 – 1E-6 (parametrized value)
K3 was set to 1E-5 for the output below
K4 = 1E-3-1E-5 (parametrized)
K4 was set to 1E-5 for the output below
K5 = 1E-2

Main Code

```
clc
clear

Time = [0 1000];
Toluene = 9.2; % initial Toluene concentration [mg/L]

[t, Y] = ode45('LuciferaseModelFunctions7', Time, [0, 70000000, 1E-1, 0,
10]);

L = Y(:,1);
C = Y(:,2);
T = Y(:,3);
E = Y(:,4);
Tout = Y(:,5);

subplot(2,3,1);
plot(t,L);
title('Luciferase concentration');
xlabel('time (min)');
ylabel('concentration (mg/L)');
hold on
subplot(2,3,2);
plot(t,C);
title('Cell count');
xlabel('time (min)');
ylabel('cell count');
hold on
subplot(2,3,3);
plot(t,T);
title('Toluene concentration');
xlabel('time (min)');
ylabel('concentration (mg/L)');
hold on
subplot(2,3,4);
plot(t,E);
title('Toluene dioxygenase concentration');
xlabel('time (min)');
ylabel('concentration (mg/L)');
hold on
subplot(2,3,5);
plot(t,Tout);
title('Extracellular toluene concentration');
xlabel('time (min)');
ylabel('concentration (mg/L)');
hold on
```

Iteration 8

Picture



Parameters

Parameter Symbol	Meaning	Units
Tin	concentration of toluene inside the cells	[mg/L]
Tout	concentration of toluene outside of the cells	[mg/L]
C	number of cells	[cell number]
L	concentration of luciferase	[mg/L]
E	concentration of toluene dioxygenase	[mg/L]

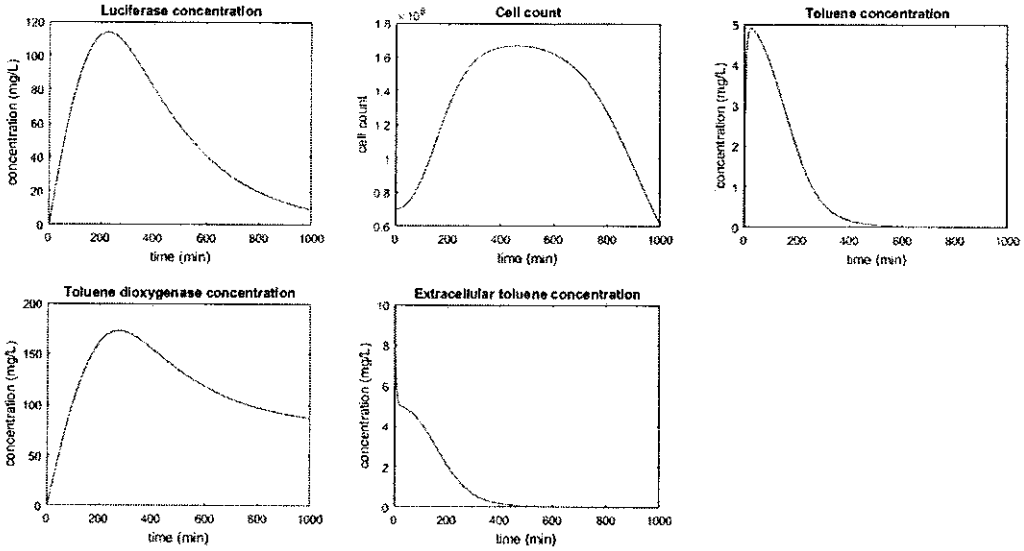
Assumptions

1. Instantaneous, homogenous distribution of toluene throughout the system
2. Cell growth is proportional to toluene consumption rate
3. Cells die at a rate proportional to the population count and inversely proportional to the toluene concentration
4. Luciferase and toluene dioxygenase follow the degradation kinetics
5. Luciferase and toluene dioxygenase do not follow the same generation kinetics
6. Degradation of toluene is directly proportional to toluene dioxygenase concentration and toluene concentration
7. Luciferase decays is proportional to the net cell change (protein is diluted when a cell divides)
8. **Toluene diffuses from outside the cells to inside the cells via Fickian diffusion**

Bolded assumption indicates what is changed for this iteration

Equations

Output


Luciferase concentration
concentration (mg/L)
time (min)
Cell count
cell count
time (min)
Toluene concentration
concentration (mg/L)
time (min)
Toluene dioxygenase concentration
concentration (mg/L)
time (min)
Extracellular toluene concentration
concentration (mg/L)
time (min)

Function Code

```
function A = LuciferaseModelFunctions8(~,Y)
A = zeros(5,1);

L = Y(1);
C = Y(2);
Tin = Y(3);
E = Y(4);
Tout = Y(5);

% Cells grow proportionally to toluene concentration
k3 = 1E-5;
k4 = 1E-5;
A(2) = k3*C*E*Tin-k4*C/Tin;

% Luciferase level
klucif = 2;    % [unitless]
Klucif = 5;        % [mg/L]

% calculate degradation rate
HalfLife = 180;      % [minutes]
AlphaL = log(2)/HalfLife;

A(1) = klucif*Tin/(Klucif+Tin)-AlphaL*L;

% Toluene level
k5 = 1E-1;
ExtoIn = k5*(Tout-Tin);

k2 = 1E-12;
A(3) = ExtoIn -k2*C*E*Tin;

% Toluene dioxygenase level
ktol = 2;
Ktol = 3;
A(4) = ktol*Tin/(Ktol+Tin) - AlphaL*L;

% Extracellular toluene
A(5) = -ExtoIn;

end
```

Equations

$$\frac{dT_{out}}{dt} = -k_5(T_{out} - T_{in})Todx$$

$$\frac{dT_{in}}{dt} = k_5(T_{out} - T_{in})Todx - k_2CET$$

$$\frac{dC}{dt} = k_3CET - k_4\frac{C}{T}$$

$$\frac{dL}{dt} = \frac{V_{m,lucif}T}{K_{m,lufic} + T} - k_1L$$

$$\frac{dE}{dt} = \frac{V_{m,tol}T}{K_{m,tol} + T} - k_1E$$

$$\frac{dTodx}{dt} = \frac{V_{m,tol}T}{K_{m,tol} + T} - k_1Todx$$

Initial Conditions

Tout(0) = 9.2 mg/L
C(0) = $7*10^8$ cells
L(0) = 0 mg/L
E(0) = 0 mg/L
Tin(0) = .001 mg/L
Todx(0) = .01 mg/L

Parameter Values
Vm,lucif = 2 (researched value)
Km,lucif = 5 mg toluene/L (researched value)
Vm,tol = 2
Km,tol = 5
K1 = log(2)/toluene dioxygenase half life = .00167
K2 = 1E-10 – 1E-12 (parametrized value)
K2 was set to 1E-12 for the output below
K3 = 1E-4 – 1E-6 (parametrized value)
K3 was set to 1E-5 for the output below
K4 = 1E-3-1E-5 (parametrized)
K4 was set to 1E-5 for the output below
K5 = 1E-2

Main Code

```
clc
clear

Time = [0 1000];
Toluene = 9.2; % initial Toluene concentration [mg/L]

[t, Y] = ode45('LuciferaseModelFunctions9', Time, [0, 70000000, 1E-3, 0, 10,
1E-2]);

L = Y(:,1);
C = Y(:,2);
Tin = Y(:,3);
E = Y(:,4);
Tout = Y(:,5);
Todx = Y(:,6);

subplot(2,3,1);
plot(t,L);
title('Luciferase concentration');
xlabel('time (min)');
ylabel('concentration (mg/L)');
hold on
subplot(2,3,2);
plot(t,C);
title('Cell count');
xlabel('time (min)');
ylabel('cell count');
hold on
subplot(2,3,3);
plot(t,Tin);
title('Toluene concentration');
xlabel('time (min)');
ylabel('concentration (mg/L)');
hold on
subplot(2,3,4);
plot(t,E);
title('Toluene dioxygenase concentration');
xlabel('time (min)');
ylabel('concentration (mg/L)');
hold on
subplot(2,3,5);
plot(t,Tout);
title('Extracellular toluene concentration');
xlabel('time (min)');
ylabel('concentration (mg/L)');
hold on
subplot(2,3,6);
plot(t,Todx);
title('TodX concentration');
xlabel('time (min)');
ylabel('concentration (mg/L)');
hold on
```