TPA VS PCA DETECTION

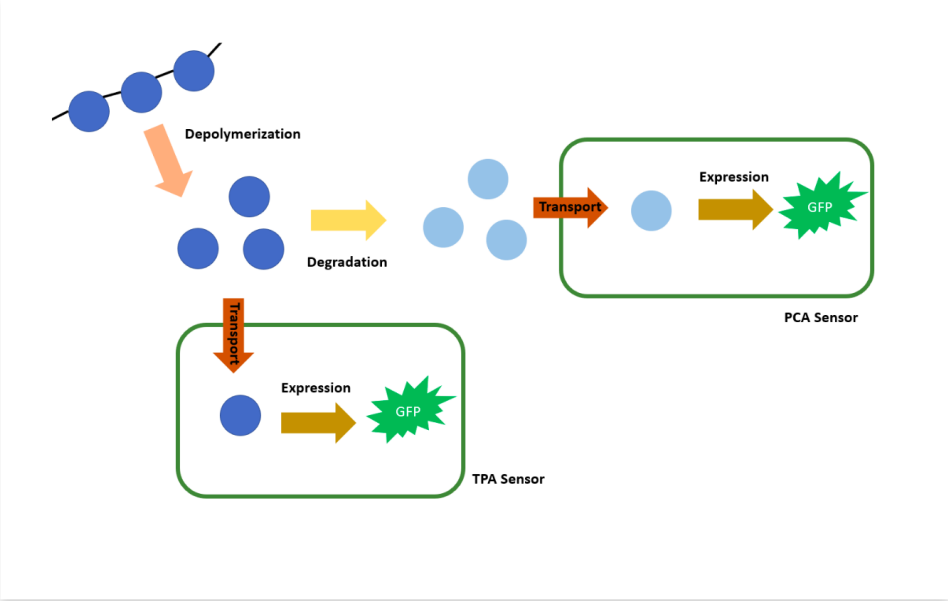
a consideration of response time and sensitivity

We found two potential biosensor systems that we could possibly use for detection of the degradation of PET, one which detected protocatechuate (PCA) and the other detecting terephthalate (TPA). To determine which sensor system was better for our needs, we used the MatLab SimBiology package to model the degradation of PET down to PCA and further to the cellular metabolite. The SimBiology files are available at this link:

Simbiology Zip File for TPA vs PCA

Simulation Setup

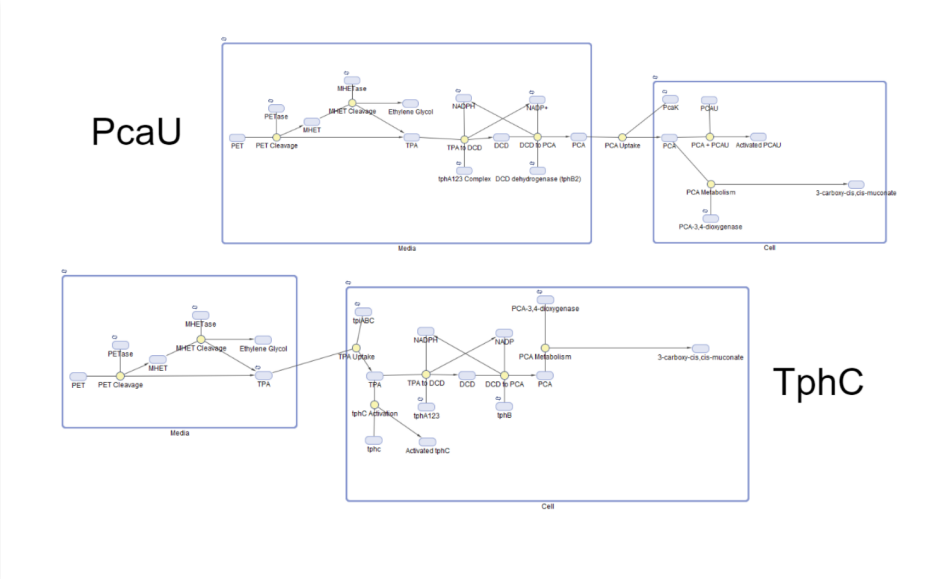
There are four major processes that occur in our sensor setup as shown below:



which are the depolymerization of PET into TPA, the degradation of TPA to PCA, the transport of PCA and TPA, and the expression of GFP from the activation of transcription factors. The parameters of the TPA and PCA sensor systems are described below:

TPA Sensor	PCA Sensor
• Uses TpiAB transporter • TphC transcriptional activator • Require no cell extracts • Low solubility of TPA in solution	• Requires cell extract for degradation • Uses PcaK transporter • PcaU transcriptional activator • Better characterized • PCA is rapidly metabolized in the cell

Using SimBiology we created two sensor systems for our degradation analysis:



Through an extensive literature search, we found the enzyme kinetics parameters and protein concentrations as listed below. We converted all values of Vmax and kcat in units of M product / (s * M protein). We used non-reversible Michaelis-Menten as our enzyme kinetics parameters except the degradation of PETase, which we approximated using our zero order enzyme kinetics equation described here and the transport of TPA and PCA by TpiAB and PcaK respectively, which followed a law of mass action kinetics.

Reaction	Parameter	Value	Source & Justification
PET Cleavage	kf (hc)	0.00463 s^-1	Calculated from Yoshida et al using a zero order rate equation. Using 50 nM of enzyme, PETase produced a total of 0.015 mM of product when fed high-crystalline PET and 0.3 mM of product when fed thin-sheet PET in 18 hours. [2]
	kf (lc)	0.0926 s^-1	
MHET Cleavage	kcat	31 s-1	Km and kcat reported by Yoshida et al. (2016) Vmax was calculated using the equation Vmax = kcat[E] using the enzyme concentration of 50 mM [2]
	Km	7.3 x 10^-6 M	
	Vmax	1.55 x 10^-6 M s^-1	
TPA to DCD	Km NAD	6 x 10^-6 M	Km values given by Fukuhara et al.. Vmax values were given by Fukuhara et al as 9.87 U/mg. Since 1 U = 1 umol / min, Vmax is 9.87 umol min^-1 mg^-1. [3]
	Km TPA	7.2 x 10^-5 M	
	Vmax	1.645 x 10^-7 mol s^-1 mg^-1	
		17.2 mol DCD s^-1 mol^-1 tphA123	Converted using the assumption of 1 L of culture. Using the mass of the enzyme to be 104.860 kDa.
DCD to PCA	Km NAD+	4.3 x 10^-5 M	Km values reported in Saller et al. The specific activity of pure protein was found to be 533 mkatal / kg protein. since 1 katal = 1 mole / second, the Vmax is 5.33 x 10^-4 mmol s^-1 mg^-1 [4]
	Km DCD	9.0 x 10^-5 M	
	Vmax	5.33 x 10^-7 mol s^-1 mg^-1	
		17.56 mol PCA s^-1 mol^-1 protein	Converted using the assumption of 1 L of culture. Using the mass of the enzyme to be 32939.02 Da.
PCA Metabolism	k1	3.5 x 10^4 M-1 s-1	Reaction of the enzyme with protocatechuic acid was too fast to analyze. 3,4- dihydroxyphenylacetic acid, were used to slow down the rate of the reaction. Reaction rate and Km values correspond to the kinetic values of the replacement molecule (Fujisawa, 1972). The paper used 3.84 mg of protein per mL of solution, which converts to 5.486 ug of protein using the molecular weight of the enzyme of 700 kDa. This translates to a concentration of 5.486 mM. The Vmax was calculated assuming that the binding to the oxygen essentially happens at the same time as binding to the substrate. This is justified by the almost irreversibility of the oxidation reaction and the fast reaction time. The k3, which is the rate of product formation, was used as kcat. Vmax was calculated using equation Vmax = kcat[E] [5]
	k-1	7.7 s-1	
	k2	2.3 x 10^5 M^-1 s^-1	
	k-2	Almost zero	
	k3	0.18 s^-1	
	Km (organic)	6.7 x 10-6 M	
	Km (O2)	1.2 x 10-6 M	
	Vmax	9.8748 x 10^-4 M s^-1	
PCA Uptake	Km	6.0 x 10^-6 M	The Km was given in Nichols & Harwood for 4-hydroxybenzoic acid. The Vmax was given as 25 nmol/min/mg of protein. [6]
	Vmax	4.167 x 10^-10 mol mg^-1 s^-1	
		0.01966 mol PCA s^-1 M^-1 protein	Converted using the assumption of 1 L of culture. Using the mass of the enzyme to be 47,177 Da.
TPA Uptake	Vmax	4.37 x 10^-11 mol mg^-1 s^-1	The TPA uptake rate in the absence of competitors was 2.62 nmol per mg of protein per min. [7]
		0.004565 mol TPA s^-1 M^-1 protein	Converted using the assumption of mmol of protein per 1 L of culture. Using the mass of the enzyme to be 34,410 (TphC) + 53,004 (TpiB) + 17,057 (TpiA) = 104,471 Da.
PcaU Activation			In Seihler et al, it is seen that with 1 mM PCA, there is 2740 Miller Units. [8,9]
TphC Activation			According to Kasai et al in 2010, beta-Galactosidase activity was 2.03 mU mg^-1 with tphC in the presence of 10 mM of TPA. [10]

Excel file with links

We ran the simulation with the following parameters:

- “600 uM” of PET added in solution

- Constant enzyme concentrations

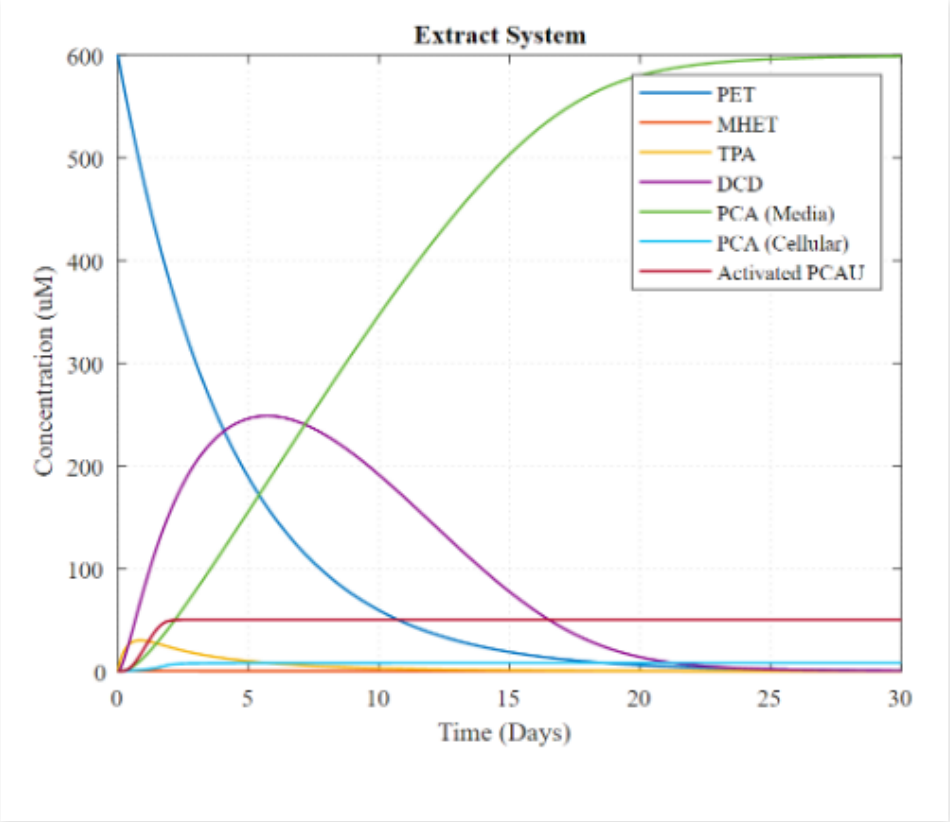
- Reversibility of reactions not considered

- Simulated rich oxygenated media with excess NADPH

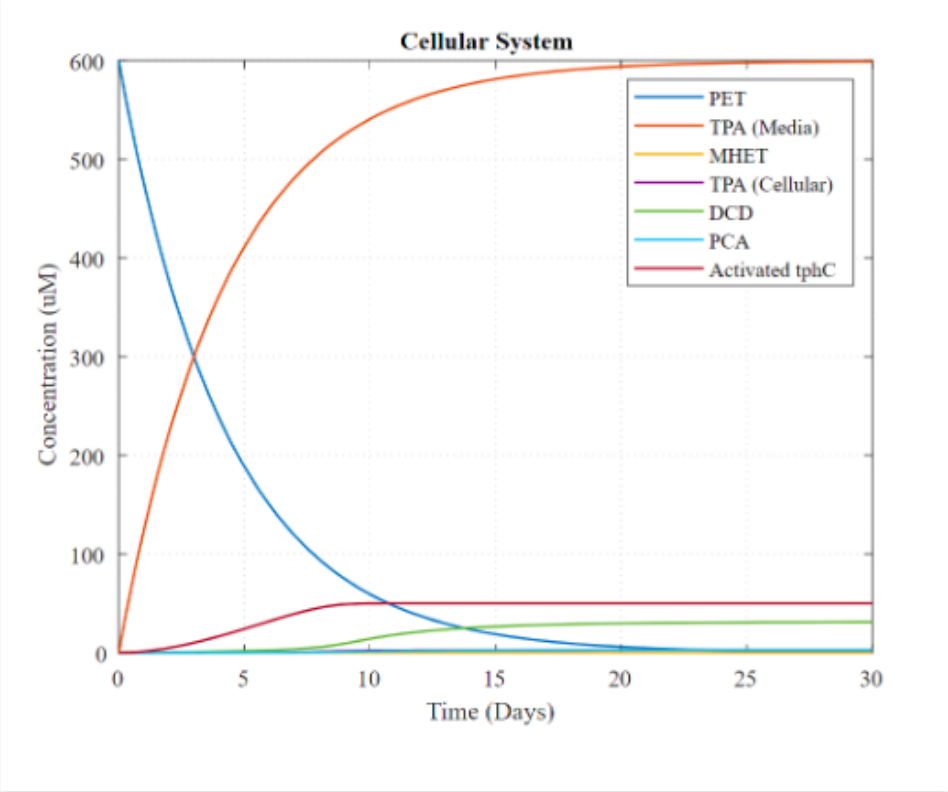
- Fixed 50 uM of transcription factor concentrations (a gross exaggeration)

- 30 Day simulation

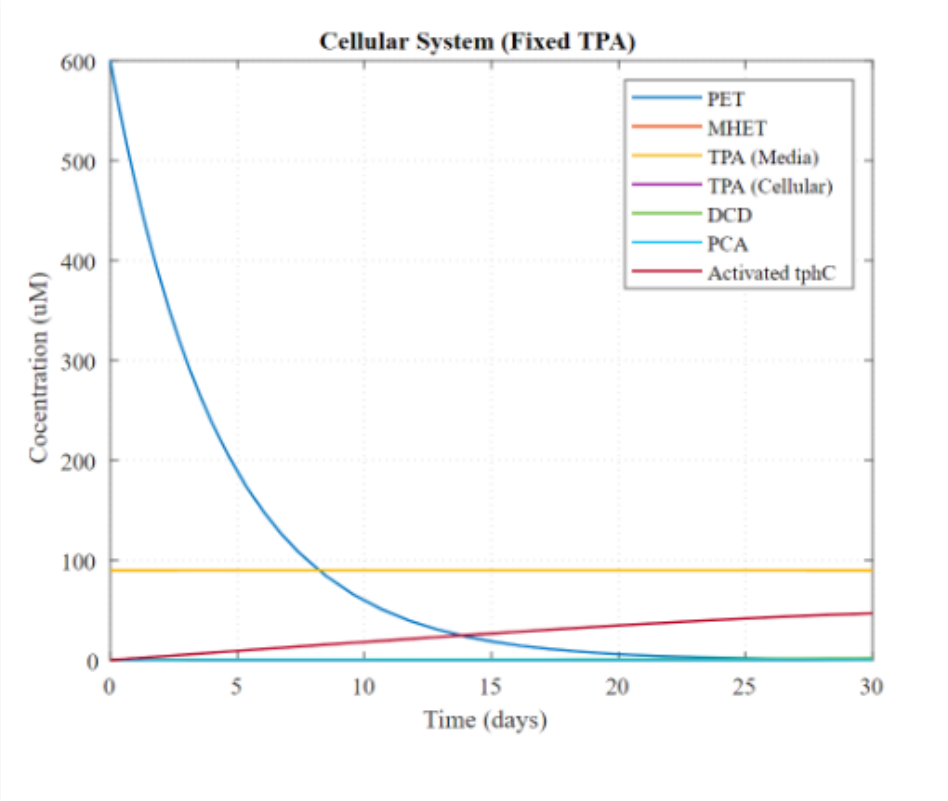
Simulation Results



In the PCA detection system, we predict that there are high levels of PCA in the media, but the rate limiting reaction in the degradation pathway is the conversion of DCD to PCA. Transport of PCA limits the activation of the PcaU. The model predicts that concentrations of PCA will exceed the solubility limit (120 uM in water), but we see that there is full activation of PcaU prior to the solubility limit being reached. There is rapid activation of PcaU at about 1.5 days.



In the TPA detection system we see an immediate increase in the concentration of TPA in the media, with a similar problem of the rate of transport limiting the activation of TphC. In this simulation, we see an increase in the time to full response to about 7 days, which is about 4x longer than the PCA based system. However, in this simulation we exceed the solubility limit of TPA (90 uM) rapidly, therefore we tweaked the model to a fixed concentration 90 uM concentration of TPA in the media.



In the fixed TPA concentration model, we see an even longer increase in the time to full response to almost 30 days, which is now 20x slower compared to the PCA based system. Therefore we concluded that for fastest response time to degradation of PET, a PCA based sensor system would be much better than a TPA based sensor system.

With regards to sensitivity, we were unable to get a clear picture from our literature search. We searched for lacZ activity which is downstream of the PCA or TPA activated promoter system. There are conflicting units of specific activity (U) and Miller Units, which cannot be converted between each other. However, we found that the detection limit of PCA is close to 1 uM while the detection

of TPA is close to 10 uM [8,10]. Therefore we concluded that the sensitivity of the PCA sensor is also better than the TPA based sensor.

Simulation Conclusions

- The biochemistry literature is of limited help when trying to acquire values

- PCA Detection System provides better response time and sensitivity vs.the TPA Detection System

- Transport across the membrane the limiting factor in both scenarios

- Increasing the expression of transport proteins the biggest challenge

- PcaK transport system is much faster and smaller than the TpiBA system

- Metabolism of PCA negligible in limiting response of PcaU system

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