DEGREDO PETRONUM

METU iGEM 2014 Team

Giant Jamboree Boston
10/31/14
Pollution

Environmental Health Problems

(Reference:
Degredo PETronum!
Plastic PET Production

Figure 3: World Plastics Production 2010
Source: PlasticEurope Market Research Group (PEMRG)
Plastic PET Production

- 265 Mtone produced each year
PLASTIC PRODUCTION / PLASTIC RECYCLING

Figure 1
Growth in Post-Consumer Plastic Bottle Recycling

Figure 2: World Plastics Production 1950-2010
Source: PlasticsEurope Market Research Group (FEMRG)
Disposal, Recycling and Energy Recovery 2012

Figure 11: Disposal, recycling and energy recovery in 2012
Source: Consultic

In 2012 plastics recycling and energy recovery rate continues to increase. (EU-27+N/CH)

Pyruvate

WHY NOT?
Why not synthetic?
PET consists polymerized units of the monomer ethylene terephthalate, with repeating $\text{C}_{10}\text{H}_8\text{O}_4$ units

Structural Analysis
PET- Pyruvate Pathways

PET (polyethylene terephthalate)

TPA (terephthalic acid)

DCD ((1R,2S)-dihydroxy-3,5-cyclohexadiene-1,4-dicarboxylic acid)

PCA (protocatechuic acid)

Catechol

2-hydroxymuconate semialdehyde

2-oxopent-4-enoate

4-hydroxy-2-oxopentanoate

Pyruvate

http://2014.igem.org/Team:METU_Turkey_project
Our Circuit Design

J23108  C.Promoter  K316003  RBS+Catechol  K1095000  Hydrolase  K1392931  Hydratase  K1392932  Aldolase  B1006  Terminator

2,3-dioxygenase
New part Bba_K1392931

2-oxopent-4-enoate + H₂O → 4-hydroxy-2-oxopentanoate

2-oxopent-4-enoate hydratase

*Escherichia Coli* BL21(DE3)
New part Bba_K1392932

4-hydroxy-2-oxoglutarate $\rightarrow$ pyruvate + glyoxylate

4-hydroxy-2-oxoglutarate aldolase

Escherichia Coli BL21(DE3)
Characterization of Catechol-2,3-dioxygenase

K1392991 = J23108 + K316003
Kill-Switch

K80800    K515004           C0040         B1006
AraC      RBS+Antiholin    Tet+LVA     Terminator

R0040     K112806           K124014         B1006
TetR       T4 Endolysin       Holin         Terminator
The issue

Arabinose present

Arabinose absent
MODELING
MODELING

Catechol → Catechol 2-3 dioxygenase → 2-hydroxymuconate semialdehyde → 2-hydroxymuconate semialdehyde hydrolase → 4-hydroxy 2-oxopentaonate → 4-hydroxy 2-oxovalerate aldolase → 2-oxopent 4-enoate hydratase → 2-oxopent 4-enoate → Pyruvate
Catechol Degredation

- Simulation of catechol diffuses by cell and degrades to pyruvate instantly

http://2014.igem.org/Team:METU_Turkey_modeling
## Parameters of Catechol Degradation

Parameters of our simulation which we used in catechol degradation to pyruvate

<table>
<thead>
<tr>
<th>Name</th>
<th>Value</th>
<th>Explanation</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1_St</td>
<td>0.04</td>
<td>promoter strength</td>
</tr>
<tr>
<td>catechol_diffusion</td>
<td>0.000076</td>
<td>catechol diffusion coefficient per cm^2</td>
</tr>
<tr>
<td>deg_p1prot</td>
<td>0.00022</td>
<td>catechol 2,3-dioxygenase degradation rate</td>
</tr>
<tr>
<td>Vm_c2h</td>
<td>930</td>
<td>catechol 2,3-dioxygenase V_max</td>
</tr>
<tr>
<td>Km_c2h</td>
<td>0.015</td>
<td>catechol 2,3-dioxygenase Km</td>
</tr>
<tr>
<td>p1_translation</td>
<td>0.0017</td>
<td>catechol 2,3-dioxygenase translation rate</td>
</tr>
<tr>
<td>Vm_2h2o</td>
<td>35</td>
<td>2-hydroxymuconate semialdehyde hydrolase V_max</td>
</tr>
<tr>
<td>Km_2h2o</td>
<td>0.017</td>
<td>2-hydroxymuconate semialdehyde hydrolase Km</td>
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<tr>
<td>deg_p2prot</td>
<td>0.0000992</td>
<td>2-hydroxymuconate semialdehyde hydrolase degradation rate</td>
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<tr>
<td>p3_translation</td>
<td>0.0017</td>
<td>2-oxoprop-4-enolate hydratase translation rate</td>
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<td>Vm_2o4h</td>
<td>450</td>
<td>2-oxoprop-4-enolate hydratase V_max</td>
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<td>Km_2o4h</td>
<td>0.000041</td>
<td>2-oxoprop-4-enolate hydratase Km</td>
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<td>p4_translation</td>
<td>0.0017</td>
<td>4-hydroxy-2-oxovalerate aldolase translation rate</td>
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<tr>
<td>Vm_4hp</td>
<td>5.6</td>
<td>4-hydroxy-2-oxovalerate aldolase V_max</td>
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<tr>
<td>Km_4hp</td>
<td>88.6</td>
<td>4-hydroxy-2-oxovalerate aldolase Km</td>
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</table>

Characterization of our Bba_K1392991 part by exposing catechol
Graphs of Kill Switch Model

1) Presence of arabinose the simulation of AraC promoter without holin and endolysin

http://2014.igem.org/Team:METU_Turkey_modeling
2) Absence of Arabinose, the simulation of cell lysis by holin and endolysin

http://2014.igem.org/Team:METU_Turkey_modeling
Parameters of our kill switch model by using AraC and TetR promoter

<table>
<thead>
<tr>
<th>Name</th>
<th>Value</th>
<th>Explanation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dif_Ara</td>
<td>7x10^{-18}</td>
<td>Arabinose Diffusion Rate</td>
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<tr>
<td>Diss_AraC</td>
<td>0.00012</td>
<td>Dissociation of AraC</td>
</tr>
<tr>
<td>As_AraC</td>
<td>0.00012</td>
<td>Association of AraC</td>
</tr>
<tr>
<td>Deg_AraC</td>
<td>0.0013833333</td>
<td>Degradation of AraC</td>
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<tr>
<td>H_Co</td>
<td>2.26</td>
<td>Hill Coefficient</td>
</tr>
<tr>
<td>Deg_mR</td>
<td>0.00288</td>
<td>Degradation of mRNA</td>
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<tr>
<td>Pro_TetR</td>
<td>0.0655x10^{-6}</td>
<td>TetR max. production rate</td>
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<tr>
<td>Rep_TetR</td>
<td>0.001667x10^{-6}</td>
<td>TetR repression coefficient</td>
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<tr>
<td>Deg_TetR</td>
<td>0.001153</td>
<td>TetR degradation rate</td>
</tr>
<tr>
<td>Cf Co TetR</td>
<td>2</td>
<td>TetR cooperativity coefficient</td>
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</table>

http://2011.igem.org/Team:St_Andrews/modelling
http://2011.igem.org/Team:ETH_Zurich/Modeling/Parameters
Achievements

2 New Parts added to the library
- K1392931
- K1392932

Composite and Characterization
- K1392991

Composite Parts added to the library
- K1392971
- K1392972
- K1392939
- K1392938
InterLab Study
1) BBa_I20260 in the pSB3K3 vector

2) BBa_J23101 + BBa_E0240 in pSB1C3 backbone

3) BBa_J23115 + BBa_E0240 in pSB1C3 backbone
Mean fluorescence intensity

A04 LB+E.coli: All 1,533.32

B01 DV1-S1: All 1,194.19
B02 DV1-S2: All 1,079.89
B03 DV1-S3: All 1,165.72

C01 DV2-S1: All 1,416.72
C02 DV2-S2: All 1,371.77
C03 DV2-S3: All 1,345.70

D01 DV3-S1: All 1,419.34
D02 DV3-S2: All 1,355.00
D03 DV3-S3: All 1,123.69
SynBio Survey!
II. METU The Day of Synthetic Biology!
PET Degradation Game
METU Development Foundation Schools

- We gave Lab education to young iGEM'ers!
- We have started, advised and helped to this year's High School Team "METUHS-Ankara"!
- We are raising new iGEM'ers!
PET Degredation Meeting in Eskişehir!
SynBio Party! #2

SynBio Party

SynBio Party

SyBio Party! #3

AUTUMN Party
Future Prospect

Genetic Modified Organism
Thank you for your attention

Special thanks to:
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R.A Çiğdem Yılmaz
R.A Handan Melike Dönertaş
R.A Side Selin Su Yirmibeşoğlu
R.A Alişan Kayabölen
CEO Burak Yılmaz
Sponsors
Team

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