Crust Away

iGEM Trento 2012
Could bacteria provide a solution?
In the air

SO\(_2\) + H\(_2\)O

H\(_2\)SO\(_3\)

H\(_2\)SO\(_4\)

Acid Rain

Bad!

Marble Surface

CaCO\(_3\)

Polylactics

H\(_2\)SO\(_4\)

Black Crust

CaSO\(_4\)

Bad!
Strictly Anaerobic

Not controllable

Not genetically tractable
Alternative Sulfate Reducing Pathway

- Aerobic
- Cost efficient
- Safe
- Controllable
- Not harming the marble
Expression

- sfGFP
- araC-pBAD
- M256I CysE
- RBS
- + GLYCEROL
- + GLUCOSE

**Graph:**
- Intensity of fluorescence (a.u.)
- Time (h)
- Bar chart showing expression intensity over time with two conditions: + GLYCEROL and + GLUCOSE.
Cysteine Production

Carbon source
Arabinose
Glycerol -   Glycerol +   Glucose -   Glucose +   Empty Cells

[Cys] (mM)

0.0500
0.0375
0.0250
0.0125
0

araC-pBAD RBS M256I CysE
OUT
IN
CYSTEINE
IPTG
GAS
BBa_K731400
LacI-lacIq
Ptac
lacO
RBS
CysDes
CysDes
Cysteine

$[\text{H}_2\text{S}]$ (mM)

H$_2$S Production

Lacl-laclq  Ptac  lacO  RBS  CysDes
H₂S Quantification
THE SRB HANDBOOK
SAFETY GUIDELINES FOR
H₂S PRODUCING BACTERIA
LacI-lacIq
CysDes
RBS
Ptac
lacO
CysDes

sfGFP
araC-pBAD
RBS
M256I CysE

CysE-sfGFP fluorescence (a.u.)

0 50 100 150 200

0 1 2 3 4 5 6

-IPTG
+IPTG

-time (h)

-IPTG
Cysteine Degradation

<table>
<thead>
<tr>
<th>CysE Induced?</th>
<th>CysDes Induced?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>Y</td>
<td>N</td>
</tr>
<tr>
<td>N</td>
<td>Y</td>
</tr>
<tr>
<td>N</td>
<td>N</td>
</tr>
</tbody>
</table>

[Cys] (mM)

![Graph showing cysteine degradation levels](image)
Cysteine Degradation

CysE Induced?  Y  Y  N  N  N
CysDes Induced?  Y  N  Y  N  N

[Cys] (mM)

0.0500
0.0375
0.0250
0.0125
0.0000

WORKS!
Denser 18 g/L agar Gel

Jelly - MOPS 6 g/L with cells

Three 12 h applications
Synthetic black crust is removed by our system.
Achievements

- Built 30 Parts
- Submitted 21 Parts
- Characterized 11 Parts
- Improved 2 Parts
- Aerobic sulfate reduction pathway
- Reconstructed artificial black crust
- Developed a new bioremediation method to clean marble
- SRB Safety Book
- Outreach Activities

Future Directions

- Optimize coexpression
- Optimize application method
- Have definitive negative controls
- Test on different stones and black crust types