Cyano Pac-man
This is the synthetic biodesalinator.
Outline

- Background
- Design
- Experiment & Results
- Human Practice
Background

South-to-North Water Transfer Project

Background

$30,000,000,000

400,000 Residents

Its present disadvantages
We are still facing the problem...

What about developing new water resources?
Background

Raw material for seawater desalination

- 96.5% Ocean
- 3.5% Other water source
The challenges

3.4~4.5 kwh/m^3

Approximately 795 miles

= The distance between Chicago and New York
#### Background

**SJTU-BioX-Shanghai**

#### Industrial biodesalination workflow

1. **Intake of seawater**
2. **Desalination**
3. **Cell-water separation**
4. **Further downstream process**
1. Photoautotrophic
2. Adaptable to diverse environments
3. High Salinity-tolerant
4. Amenable to Genetic manipulation.

**Cyanobacteria—the chassis Organism**
*Synechosystis* sp. PCC6803
Outline

- Background
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Design

- Biodesalination driver
- Biodesalination controller
- Construction
- Biodesalination process

Experiment & Results

- Transformation
- Expression
- Biodesalination Assay
Design: Biodesalination Driver

Retinal isomerization

Yifan Song, and M. R. Gunner PNAS 2014
Design: Biodesalination Driver

1. Starvation

2. Halorhodopsin Expression

Na⁺/H⁺ antiporter

P-type Na⁺ ATPase

Halorhodopsin

Na⁺ channels

Active sodium export

Desalination under starvation

ATP

ADP + Pᵢ

H⁺
Design: Biodesalination controller

Green-light inducible promoter from *Synechosystis sp.* PCC 6803

No green light

Under green light
The design of **Pdark**

**CcaR Binding site**

**J23107**

**Pdark**

**-35box**

**-10 box**

From 2013 SJTU-BioX-Shanghai
Design: Biodesalination Controller

SJTU-BioX-Shanghai

Modified Promoter: Pdark

Under natural light

Under darkness
Design: Constructions

PcpcG2 –HR (BBa_K1642010)

Pdarker –HR (BBa_K1642011)

Plasmid backbone: pBluescript KS(+)  Locus: slr0168
Design: Biodesalination Process

Test the Biodesalination ability of engineered Strain

Controlled by PcpcG2

Growth stage  Induction stage (Starvation stage)  Working stage

Controlled by Pdark
E&R: Transformation

SJTU-BioX-Shanghai

Day
0  Inoculation
2  Electroporation
10 Picking colonies
20 5 generations of Liquid Aggregation

PcpcG2-HR

Pdark-HR
Immunocytochemistry

Wild Type | Pdark-HR | PcpcG2-HR

Primary antibody: **anti-His mAb**
Secondary antibody: **goat anti-mouse IgG Alexa Fluor 488**
E&R: Biodesalination Assay

Seawater: 30g/L NaCl

BG11 medium + 22g/L NaCl

Sodium: 9.6 g/L
Chloride: 17.6 g/L

Growth stage

Induction stage
(Starvation stage)

Working stage

0 hour

10mM Retinal&
22g/L NaCl
E&R: Biodesalination Assay

Sodium: Ion Chromatography

Chloride: Inductively Coupled Plasma–atomic Emission Spectrometry (ICP-AES)
E&R: Biodesalination Assay

Assay on PecpG2-HR

**Sodium**

![Sodium Ion Concentration vs. Time](chart1)

**Chloride**

![Chloride Ion Concentration vs. Time](chart2)

Regain of energy
Assay on Pdark-HR

Reduction by 20% at 10h

Sodium

Chloride
Alternation: natural light and darkness

- Induction Stage (Starvation Stage)
- Working Stage
- Starvation Stage
- Working Stage
- Starvation Stage

...
Summary

### Design

**Driver:** Halorhodopsin  
**Controller:** Pdark  
**Process:** Three stage

### Result

Engineered cyanobacteria with **biodesalination** ability based on the expression of **halorhodopsin** and process controlled by PcpcG2 and **Pdark**.
Future Work

1. **Retinal** synthesis in PCC 6803.
3. Optimization of biodesalination process—
   length of growth stage and Induction stage;
   light intensity.
4. Construction of higher salinity tolerant stain.
5. Testing desalination ability in real seawater.
Outline

Background

Design

Experiment & Results

Human Practice
1. Not overthrowing the desalination success
2. Fitting the goal of lowering the energy consumption
3. Reducing bio-risk
Cell-water separation

Inspiration: algae-controlling

Prof. He working on algae controlling in Taihu
Cell-water separation

Dissolved air floatation

1. Non-chemical
2. Efficient
3. Mature

Valuable natural components & product from algae bio-mass:
1. Natural pigment
2. Algal bile protein
3. Extracellular polysaccharide
4. Fertilizer & Fuel
Cell-water separation

Future work guide

If single use still ...
- **Further goal: Increasing efficiency**
- Method: Working on the natural vesicle

If long-term use ...
- **Further goal: Immobilization**
- Method: Working on the uprising line of desalination essay and Immobilization (collaborated Nanjing iGEM team)
Further downstream process
Inspiration: Present desalination tech

Hangzhou Water Treatment Technology Development Center working on RO membrane production and relating technology development.
Reverse osmotic method

Under no external force

Low salinity → High salinity

RO membrane

Under the external force

Low salinity ← High salinity

RO pressure

Main Energy form

Energy cost (lower)

Product water quality

99% VS 18%

Pretreatment requirement

Seawater RO

Our project

Human Practice

SJTU-BioX-Shanghai
Further downstream process & Reverse osmotic method

1. Product water quality
2. Efficiency
3. Earlier application

Further downstream process

Future work guide

- Further goal: To really connect these two methods

- Method:
  1. Virtualization of RO desalination
  2. Testing using real seawater and include all the regular testing ions and particles after cell-water separation
Safety approach

1. Double duty of industrial desalination flow

<table>
<thead>
<tr>
<th>The process step</th>
<th>The sub product it is removing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell-water separation</td>
<td>Most of the cyanobacteria, part of biomolecule</td>
</tr>
<tr>
<td>RO Pretreatment</td>
<td>The remaining cyanobacteria, biomolecules and most of the ions</td>
</tr>
<tr>
<td>RO method</td>
<td>Anything left behind beside water molecule</td>
</tr>
</tbody>
</table>

2. Supervising spot establishing

<table>
<thead>
<tr>
<th>Test point</th>
<th>Test Item</th>
<th>Reasons and further use of the data</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intake</td>
<td>GB 3097-1997</td>
<td>The seawater properties changes during different seasons and months. We cannot change the intake spot to adjust, yet, we certainly can adjust the pretreatment to make sure this doesn’t influence the outcome.</td>
</tr>
<tr>
<td>Before bio-desalination</td>
<td>Main: CFU Inorganic nitrogen Reactive phosphate</td>
<td>Bio-process appreciated a rich nutrition environment without any competitive organism. To test how rich the water is will help to adjust the growing stage of cyanobacteria.</td>
</tr>
<tr>
<td>After cell-water separation</td>
<td>Main: CFU Inorganic nitrogen Reactive phosphate</td>
<td>This test is to ensure most of the cyanobacteria and biomolecule is removed.</td>
</tr>
<tr>
<td>Before RO membrane method</td>
<td>GB 5749-2006</td>
<td>Especially the element which damage the RO membrane</td>
</tr>
<tr>
<td>Outcome</td>
<td>Boron GB 5749-2006</td>
<td>Product check!</td>
</tr>
</tbody>
</table>
The collaboration study with Nanjing iGEM

Proposing the new seawater heavy metal recovery system

- Offsetting the brine’s effect on environment in the way of total environmental assess.
- Creating a less environmental affecting and quicker restoration system

Public

Water shortage report in China

1. Circumstance
2. Reasons
• The government support investigation on law aspect
• The important fact to gain government support
• Fitting the local needs

Shanghai water supply authority
Interaction
with other iGEMers

Meetup schedule
• 4.11: SCUT-Champion-Park
• 4.19: China_Tongji, NJAU_China
• 6.8: ITB_INDONESIA, China_Tongji, NYU_Shanghai
• 7.11: SYSU_China
• 7.14: WHU-China
• 8.6: ZJU-China
• 8.8: NYU_Shanghai, China_Tongji, ZJU-China, XJTLU-China, Fudan
• 8.26: Tsinghua

Survey list
"Results and transparency in iGEM wikis" from iGEM Stockholm
• "Questionnaire for previous iGEMer" from Tianjin-iGEM team
• "iGEM Rhizi Tutorial" from Paris Bettencourt
• "iShare: All-round Resource Sharing Proposition" from iGEM Nankai
• "survey about wiki" from NEFU_China
• "Cloning Survey" from Stanford-Brown 2015 iGEM
• "BaContraception" from Team UI_Indonesia
Human Practice
SJTU-BioX-Shanghai

Team
Human Practice
SJTU-BioX-Shanghai

Team

Lin He
Gang Ma
Yushu Wang
Manxi Zhu
Shajia Afrin
Md. Rezaul Islam Khan
Cyanobacteria related:
Prof. Peimin Prof. Shengbin He,

Desalination related:
Hangzhou Water Treatment Technology Development Center
and their vice director, Hongqiang Li

Government related:
Prof. Xi Wang, Shanghai Water Supply Authority,

Other:
Nanjing iGEM team, Ying Chen, Lu li
Intake of seawater → Desalination → Cell-water separation → Further downstream process

Cyano Pac-man

SJTU-BioX-Shanghai

Thank you!