ALKALINE CELLULASE
DREAM FACTORY

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CHINA

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CONTENTS:

1. Background
2. Project & Design
3. Results
4. Human Practice
PART 1:

BACKGROUND

- Paper recycling; Deinking
- Environment and Sustainable Development
Chinese economy is developing rapidly
However, our environment is **deteriorating**......

The polluted river in Rural China can be seen everywhere!
Waste paper recycling industry
Use **toxic chemicals** during the deinking

Simple and crude equipment

Pour the waste water
Into the river **directly!**
The rise of **synthetic biology** provides a new way to solve this type of environmental question.

We had made an incredible *E. coli*

---- **“Alkaline Cellulase Dream Factory”**

To reduce the use of harmful chemicals.
PART 1:

Project & Design

Yiting LUO

- Four systems of the Dream factory
Alkaline Cellulase
-for the release of ink from the fiber surface by partial hydrolysis of carbohydrate molecules

Mdfa Antiporter
-resistance to alkaline wastewater

Kil
-transportation system, enhance the secretion of alkaline cellulase

CCDB
-Suicide system
Mechanism of hydrolyzing internal $\beta$-1,4-$\beta$-bonds in cellulose catalyzed by endoglucanase (Brisan et al, 1998)
Release of ink from the fiber surface by partial hydrolysis of carbohydrate molecules.

Alkaline Cellulase

endo-(1,4)-β-D-glucanase (EC 3.2.1.4)
Mdfa Antiporter

Normal bacteria

Alkaline tolerance bacteria

Alterations of cell membrane
Remodeling of metabolic patterns

Active inward transporters of protons
Mdfa Antiporter

Couple the inward movement of protons to the outward of Na+.

Maintenance of a stable cytoplasmic pH under conditions of alkaline stress.

Kil gene

Increase the permeability of cell membrane
For secretion of alkaline cellulase

1. Tong Q, Zhang H T, Yang Y G, Mediated by Kil protein of Escherichia coli secretory expression system (China Academy of Sciences in Shanghai Institute of Biological Engineering Research Center, Shanghai 200233)
**CCDB** (control of cell division death protein B) - *Suicide system*

Cell killing by the ccdB protein involves poisoning of DNA-topoisomerase II complexes.

Suicide system can be induced by the addition of the chemical IPTG.

[Diagram showing the process of ccdB induced by IPTG and its effect on Gyrase.]
Integration
FOUR PARTS

Alkaline Cellulase Dream Factory

Super pSB1C3

6419bp

pMB1

CarmR

CarmR

452x60 to 649x225

452x60 to 649x225

452x60 to 649x225

452x60 to 649x225

452x60 to 649x225

452x60 to 649x225

452x60 to 649x225

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452x60 to 649x225
PART 3:

Results

- Four systems of the Dream factory
- Deinking Test
Our Project has four parts:

- Alkaline Cellulase
- Mdfa Antiporter
- ccdB Suicide System
- Kil Protein
Alkaline Cellulase

1. Use two methods to detect cellulase activity.

Figure 1. Using CMC-Na DNS method for detection of Alkaline Cellulase

Figure 2. Transparent zones produced by E.coli on CMC-containing solid plate

Cellulase can degrade cellulose.
Alkaline Cellulase

2. The optimum reaction pH and pH stability pH=9.0

3. Research on temperature stability of Alkaline Cellulase

Optimum reaction pH is 9.0. Alkaline conditions with strong stability.

Optimum temperature is 50 °C. Ca^{2+} can improve stability.
Mdfa Antiporter

Growth contrast in LB plate medium with different pH.

Mdfa : promoter+RBS+Mdfa
control: promoter+RBS
Mdfa Antiporter

Chart of OD value of Mdfa device and control group in different pH liquid culture
Mdfa device: promoter+RBS+Mdfa
control: promoter+RBS
**kil protein**

**Determinination of extracellular enzyme activity.**

**Kil can enhance the secretion of alkaline cellulase.**

1. Tong Q, Zhang H T, Yang Y G, Mediated by Kil protein of Escherichia coli secretory expression system (China Academy of Sciences in Shanghai Institute of Biological Engineering Research Center, Shanghai 200233)
CCDB suicide system

Our *E. coli* can’t grow in the LB plate medium with IPTG
1. Alkaline Cellulase
- Cellulase can successfully degrade cellulose in this project.

2. Mdfa Antiporter
- Mdfa helping tolerate the high pH conditions in a range from pH 9.0 to PH 9.5.

3. Kil protein
- Kil can enhance the secretion of Alkaline Cellulase.

4. ccdB suicides system
- Safety precaution
- The E.coil is under effective control by the ccdB system.

Conclusion
The application of the “Dream factory”

Deinking Test

1. We marked on the same piece of paper with water ink before Deinking Experiment.

2. We cut the paper and put them in the control (bacteria liquid without Alkaline cellulase) and fermentation liquid of the “Dream factory”.
It works!

Experiment result showed that the "Alkaline cellulase dream factory" can **effectively** promote the deinking efficiency for waste paper.
PART 4:

Human Practice & Our Achievements

Yongyi WANG

- Policy and practice
- Our achievements
- Acknowledgement
Policy and practice

• Propagandizing igem in Cultural Festival
• iGEM High School *Meetup* in Hongkong
• The Communication with SUSTC
• Visiting Paper Mill
• The questionnaire
Propagandizing iGEM in Cultural Festival on campus

interacted participants with games

A happy and successful propaganda...
iGEM High School Meetup in Hongkong

conducted academic exchanges

always excited...
The Communication with SUSTC–iGEM

Communicating...

Group photo of SZU & SUSTC iGEMers
Visiting private Paper Mill

The most meaningful tour

Al$_2$(SO$_4$)$_3$

out of recognition

always curious...

practical application
Our questionnaire

The recognition of Alkaline Cellulase in application

The attitudes towards our project replied in industrial production
1. Understanding of Alkaline Cellulase
   - Synthetic Biology & iGEM
   - Questionnaire
   - Most supporting our project in application

2. Synthetic Biology & iGEM
   - Interest & Activity
   - Propaganda
   - Generalize our Alkaline Cellulase Dream Factory

3. Communication
   - High school meetup in HK
   - Share with SUSTC-iGEM
   - Academic exchanges & shares

4. Paper Mill Visit
   - The Papermill
   - Meaningful tour
   - Practical Application
   - Alkaline Cellulase in deinking

Sustainable development
Environmental protection
Our achievements:

- Collobrated with iGEM teams:
  senior high school iGEM meetup and SUSTC-iGEM

- Our biobricks:

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<th>Type</th>
<th>Description</th>
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<td>Protein Domain</td>
<td>A kind of secretory protein named kil</td>
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<td>BBa_K1350002</td>
<td>Generator</td>
<td>Alkaline Cellulase</td>
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<td>BBa_K1350003</td>
<td>Device</td>
<td>Ccpp E.coli suicide IPG</td>
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- Improved an exciting part:
  BBa_K1350007  Cel5A, endoglucanase (BBa_K805011 Improved)

- Help to ETH Zurich
  ETH Zurich invited us to participate in their survey related to complexity. As feedback, ETH Zurich gave us a badge.
Acknowledgement

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Thanks

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