BACKGROUND

Increased demand

Increased copper production
Consequence of industrial production processes of copper: A lot of sewage containing Copper Ion.
WHAT’S WORSE?

Other contaminants coupled with copper make the sewage More Toxic.

Main Common Pollutants

$\text{Cu}^{2+}$  $\text{CN}^{-}$  $\text{F}^{-}$
CURRENT TREATMENTS

Inefficient
Complex
Expensive
Secondary Pollution

No Perfect Treatment!
E. kungfu

absorb

degrade

detoxify
**E. kungfu**

太极 (Tai-Chi), a representation of Chinese Kung Fu

阳 (yang)
Retracting valuable resources
(copper ions)

阴 (yin)
Defeat bad guys
(copper, cyanide, fluoride)
NOVEL METHOD - *E. kungfu*

*E. kungfu* form biofilm on RBC

Discs rotate

*E. kungfu* dispose sewage efficiently

Rotating Biological Contactor (RBC)

- Highly capable
- Highly efficient
- Low cost
- Low secondary pollution
work system

pCusC  ompC/oprF  pCusC
Copper-sensitive promoter  Outer-membrane protein

UV

pET28a
work system

detoxifying the fluoride
work system

degradating the cyanide
Surface display system

Cell display of peptides or proteins on the surface of microorganism
kill switch
Report system

pCusC

CII

PRE

Copper-sensitive promoter

Cl

PR

GFP+ssrA

RFP+ssrA

Report system
Design

E. worker

E. instructor

Design work system

kill switch

work system

report system
Simulating the biological process of E. instructor to assure the feasibility

Fig. 1,2 DDEs Simulation Results
ROBUSTNESS AND SENSITIVITY ANALYSIS

Testing how the parameter $\tau$ (time delay) affect the output

Fig. 3,4 The effect of parameter $\tau$ in the unstable state and stable state
ROBUSTNESS AND SENSITIVITY ANALYSIS

The fluorescent output being extremely sensitive to the transcription rate

Fig. 5 The Fluorescent Output is Extremely Sensitive to the Transcription Rate

Fig. 6, 7 The Fluorescent Intensity Changes Caused by Different Transcription Rate
CIRCUIT IMPROVEMENT

Improving the circuit by providing the suggestion of changing the promoter.

Fig. 8 Gradient Analysis

Fig. 9 Promoter Test
The copper sensitive promoter pPcoA

pPcoA promoter test

Relative Fluorescent Intensity

concentration of Cu$^{2+}$ (mM)
Characterization of OprF and FlA

SDS-PAGE test of purified recombinant OprF and FlA protein
Immunofluorescence analysis of OprF displayed on *E. coli*.
Cu\textsuperscript{2+} adsorption capacity assay

The time-absorbance curve shows the successful immobilization of Cu\textsuperscript{2+}
Cu$^{2+}$ adsorption capacity assay

Bacteria + 1mg/ml CuSO$_4$
12hrs incubate then pellet
E.worker WT control

then resuspension+BCO

The time-absorbance curve shows the successful immobilization of Cu$^{2+}$
Growth curve-OprF

- oprF-GS-CBP+20mg/L Cu2+
- oprF-CBP+20mg/L Cu2+
- vector+20mg/L Cu2+
- oprF-GS-CBP+80mg/L Cu2+
- oprF-CBP+80mg/L Cu2+
- vector+80mg/L Cu2+

Works
Growth curve-FIA

- Vector-10mg/L
- Vector-300mg/L
- Vector-600mg/L
- FLA-10mg/L
- FLA-300mg/L
- FLA-600mg/L

Graph showing growth over time for different concentrations.
TOOLKIT: INTRODUCTION

Rotating Biological Contactor (RBC):

- Secondary treatment process
- Biofilms: assimilating the organic materials and other pollutants
- Aeration: facilitating the degradation of the pollutants
Advantages:

Relative low energy consumption.

Simple operation and maintenance.

Successive treatment of the influent contaminants.

Redesign the Traditional RBC!
• the bottom of the reaction tank is **concaved**
• reserve an **aperture** on the side of the sedimentation tank
• add a **small drawer** on the side of water outlet
TOOLKIT: DESIGN

Real model show:
PART 1. Team Visit to a Copper Smeltery

Daye Nonferrous Metal Corporation
5\textsuperscript{th} largest copper smeltery in China

What we learned:
Problems of sewage treatment \textit{currently used} in industry
PART 1. Team Visit to a Copper Smeltery

- Introduced iGEM and our project
- Visited the whole factory
- Know about sewage treatment currently used in the factory
PART 1. Team Visit to a Copper Smeltery

Method currently used:
Step 1: Neutralization
Step 2: Sedimentation
Step 3: Filterability of sludge

Advantage: low cost
Disadvantage: time consuming, requires large area

We have faith in *E. kungfu* to help conquer the challenge!
PART 2: The Human Practice Survey

Survey on public awareness of the effective water treatment in their daily life


PART 3: Expert Interviewing

Interview a professor in bioethics

PART 4: Assisting HZAU to hold the CCiC

PART 5: Attending the meeting-up held by NCTU

Collaboration & Communication
PART 6: Designing a fascinating game
FUTURE PLAN

Recycle Copper

Cu^{2+} → Cu
FUTURE PLAN

Replace the sedimentation step with our RBC

Sedimentation in water purification station in Daye Nonferrous Metal Corporation

Rotating Biological Contactor (RBC)
ACHIEVEMENTS

✓ Improved and submitted a part (oprF)
✓ Helped other iGEM team by constructing a part (HZAU-CHINA)
✓ Addressed big questions in sustainability and safety via smeltery visiting, interview and social survey
✓ Experimentally validate, document and submitted four new parts (oprF-GS-CBP/ oprF-CBP/ ompC/ RTS)

WE DESERVE GOLD!
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BRAND

Brand Corporation

Friend teams
We are HUST-China team
From Huazhong University of Sci. & Tech.