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论文题目：基于双荧光传感系统的 PFAS 检测及降解性能研究

Study on Simple Detection and  
Degradation of PFAS Based on a  
Dual-Fluorescence System

# Study on Simple Detection and Degradation of PFAS Based on a Dual-Fluorescence System

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**【Abstract】** Per- and polyfluoroalkyl substances (PFAS) are a class of pollutants with extremely high environmental persistence and biological toxicity. They pose severe threats to water and ecosystems due to their high water solubility and strong mobility. In order to detect and degrade PFAS efficiently, this study constructed an engineered *Escherichia coli* BL21-T3 strain with integrated “detection-degradation” functions based on synthetic biology principles. This strain enabled visual detection and degradation of PFAS by activating the expression of dehalogenase DhaA and GFP via the PFAS-sensitive promoter *prmA*. A fluoride ion riboswitch was utilized to regulate the expression of transport protein CrcB and mCherry, thereby facilitating the export of fluoride ions (the degradation products) and the formation of  $\text{CaF}_2$  precipitates. Additionally, an arabinose-inducible suicide system (CcdB) was introduced to ensure biosafety. Experimental results showed that the engineered strain could grow normally under high PFAS concentrations and exhibit specific fluorescent responses. Upon adding  $\text{Ca}^{2+}$  to its supernatant, white  $\text{CaF}_2$  precipitates formed, consistent with experimental expectations. These findings confirmed the successful construction of the engineered strain, which achieved PFAS detection and degradation via a dual-fluorescence system and holds promising application potential.

**【Keywords】** PFAS; BL21-T3; Synthetic Biology; Dehalogenase DhaA; Fluoride ion transporter CrcB

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## Introduction

Per- and Polyfluoroalkyl Substances(PFAS), a kind of contaminant that has the peculiarity of “permanent compound”, has been widely detected in global water, soil, etc. Due to its characteristic of environmental persistence, bio-accumulation and toxicity, forming a long-term threat to the ecological stability and human health. Particularly, short-chain PFAS has high water solubleness and migration ability, which make it easier to break through environmental barriers and enter the drinking water circulation, becoming the key of present water pollution treatment. Recent studies show that PFAS can cause unexpected ecological effects, e.g promoting antibiotic resistant gene in *E.coli* to transfer by increasing the permeability of the cell membrane, thus leading to the overflow of antibiotic resistance and intensify the resistance of microorganisms in polluted environment<sup>[1]</sup>.

Current PFAS dealing methods, e.g. physical adsorption and advanced oxidation, have common problems such as high cost, incomplete degradation and easily causing secondary pollution, which make it hard to satisfy the need of efficient, environmental, financial and sustainable treatment. With the fast development of synthetic biological techniques, new access to constructing an engineering microorganism with special functions to realise the integrated ‘detection-degradation-safety control’ solution has appeared.

Hereby, this research aimed to tackle the key issue in PFAS pollution treatment, selected *E.coli* BL21(DE3) as the chassis strain, combined components like special promoter, dehalogenase, F<sup>-</sup> transportation protein, fluorescence protein, suicide system, etc., designed and constructed the engineered bacteria strain BL21-T3. The bacteria goals to realise PFAS visible monitor and efficient degradation while guaranteeing the biological security of engineering bacteria through the integrated “detection-degradation-safety control” treatment, offering new technique choices to low cost and eco-friendly water PFAS treatment.

## 1. Background

Due to PFAS’ high environmental stability and the damage it can cause to human body, people have widely studied its detection and repairment strategies. Traditional analytical methods, e.g. Liquid Chromatography(LC), have become the mainstream technique of PFAS quantify as a result of its significant delicacy and specificity<sup>[2]</sup>.

Nevertheless, such methods relies on precise equipment and complicated pre-management on samples, restricting its practical use in real-time monitoring, and is consequently not generally suitable. After that, biological detection system, which takes advantage of the microorganisms' response to PFAS, offers us a new way to examine PFAS. For instance, there has already been a research that applied engineering transformation to *Rhodococcus jostii* RHA1, allowing it to express mRFP under the control of PFAS induced *prmA* promoter. Tests have shown that the engineering bacteria can increase its fluorescence intensity three times at a PFOA concentration of 100  $\mu\text{M}$ , compared to wild types<sup>[3]</sup>. Despite the potential the system showcases, it lacks integrated degradation capabilities and still requires the following steps to handle it. Because of the high stability of C-F bonds, the microbiological degradation of PFAS is facing challenges as before<sup>[4]</sup>. Furthermore, natural microorganisms' enzymes often has problems like limited substrate range or the demand to cofactors, which emphasizes the need to engineering signals with broader spectrum specificity.

Using special riboswitch and suicide gene to construct synthetic biological components can effectively tackle this problem. For example,  $\text{F}^-$  riboswitch is used to respond to  $\text{F}^-$ , the common by-product of PFAS degradation, in turn regulating genetic expression<sup>[5]</sup>. CcdB toxin-antitoxin system can lead to cell death when a certain inducer is scarce, relieving our worry of releasing engineering microorganisms into the environment<sup>[6]</sup>.

So far, our attempt to integrate detection and degradation functions into a single microorganism system is still in infancy. Most of the existing systems place their emphasis on a particular step in sensing or decomposing PFAS, but can't realise the synergy of the two aspects. For instance, *Gordonia* is confirmed to be able to degrade fluoropolymer sulfonate but isn't built-in with detection module<sup>[7]</sup>, while a kind of engineering *E. coli* can excrete  $\text{F}^-$  based on CrcB transportation protein but isn't integrated with PFAS responding components or systematic degradation signals<sup>[8]</sup>. It is clearly that the "detection" and "degradation" process in present PFAS treatment plans are quite separated and cannot form an integrated technical system with precise contaminant detection, effective degradation and environmental risk control, which is the urgent problem of research in this field. In this background, synthetic biology provides us a new access to breaking through the mentioned bottleneck. By designing the genetic circuit modularly and combining the pollutant detection component,

functional enzyme unit and biological safety control module, we can get real-time feedback of the dynamic change of the contaminant concentration and avoid potential ecological risk caused by engineering microorganisms' escape from the environment through security mechanism.

As a result, this research focused on establishing a engineering microorganism system with both three functions of “detection-degradation-safety control”, offering an efficient, environmental and steerable new solution to short-chain PFAS pollution treatment.

## 2. Solution

### 2.1 Key Parts for PFAS Detection and Degradation

(1) PFAS dehalogenase gene *dhaA*: Derived from *Mycobacterium tuberculosis* H37Rv, it cleaves carbon-halogen bonds through a bimolecular nucleophilic substitution reaction mechanism, catalyzing the dehalogenation of short-chain halogenated alkanes.

(2) Fluoride ion transporter gene *creB*: Derived from *Escherichia coli* K-12 subsp. MG1655, it functions as a fluoride ion transport channel, enabling rapid efflux of intracellular fluoride ions.

(3) *prmA*-RBS: A PFAS-sensitive promoter and ribosome binding site that enables high *dhaA* expression.

(4) sGFP: Superfolder GFP is a molecularly engineered variant of GFP that exhibits higher fluorescence intensity and greater detection sensitivity than wild-type GFP.

(5) Fluoride Ion Riboswitch (RBS): As an RNA regulatory element, this component modulates downstream gene expression by directly binding to fluoride ions ( $F^-$ ). Under low fluoride concentrations, the riboswitch assumes a “closed” conformation, thereby inhibiting transcription or translation of downstream genes. When fluoride ion concentration increases,  $F^-$  directly binds to the aptamer domain of the riboswitch, inducing conformational changes that release the inhibitory state and subsequently activate downstream gene expression.

(6) mCherry: A widely used red fluorescent protein (RFP) derived from *Discosoma striata*, it belongs to the monomeric fluorescent protein family. It can be utilized for visual monitoring of gene expression (e.g., promoter activity, transcription

efficiency) and serves as an output signal for genetic circuits.

(7) *araC-ccdB*: A suicide system regulated by the L-arabinose operon.

## 2.2 Design for PFAS Detection and Degradation Pathway

In this study, the promoter responsive to PFAS was used to initiate the expression of the PFAS dehalogenase gene (*dhaA*) and the GFP gene. To avoid interference with their respective functions, appropriate linkers and P2A self-cleavage connectors were added between them. Thus, the detection of green fluorescence in the cells indicates the release of DhaA. When the PFAS concentration in the environment is high, the *prmA* promoter is activated, initiating the expression of the *dhaA* gene (which encodes PFAS dehalogenase) and the gene encoding GFP simultaneously. This causes the engineered bacteria to emit green fluorescence under excitation light. In the absence of PFAS in the environment, the downstream genes remain unexpressed.

When no  $F^-$  are bound, the  $F^-$  riboswitch forms a hairpin structure, blocking the translation process. When  $F^-$  are present in the environment, the ions bind to the conserved stem-loop region, thereby mediating the unfolding of the hairpin structure, then activating the expression of the  $F^-$  transporter CrcB. This reduces the intracellular  $F^-$  concentration, ensuring the bacteria can survive normally even under relatively high  $F^-$  concentrations. Meanwhile, the expression of mCherry protein is initiated, enabling real-time monitoring of PFAS degradation by the engineered bacteria. The expression of mCherry was also activated.

Meanwhile, to ensure biosafety, a suicide mechanism regulated by the arabinose operon was introduced. In the absence of arabinose, the AraC protein (expressed by the *araC* gene) binds to DNA, thereby inhibiting the expression of the *ccdB* gene. When the arabinose concentration in the environment is low, the expression of the *ccdB* gene remains repressed. As the arabinose concentration increases, arabinose binds to the AraC protein, activating the pBAD promoter and thus promoting the expression of the *ccdB* gene, ultimately achieving bacterial self-destruction. As a specific DNA replication inhibitor, CcdB can induce bacterial suicide without causing bacterial lysis. Therefore, activation of this “kill switch” does not harm native microorganisms.

## 2.3 Project Innovations

Unlike traditional detection and degradation systems, this project simultaneously controls three functional modules. The engineered bacteria can autonomously activate

detection, degradation modules, and system self-destruction under specific conditions. The project’s innovations include: First, integrated functional integration enabling coordinated linkage of “detection-degradation-safety control”. Second, a dual-fluorescence system design for dynamic tracking and functional validation of the degradation process, eliminating complex instrument testing procedures. Finally, the engineered bacteria can tolerate high concentrations and exhibit significantly higher sensitivity to short-chain PFAS compared to long-chain PFAS, meeting the treatment requirements of water bodies with high short-chain PFAS contamination.

### 3.Materials and Methods

#### 3.1 Materials

##### 3.1.1 Strains and Plasmids

The chassis strain we used is *E. coli* BL21 (DE3), DH5 $\alpha$  is used for efficient amplification of target plasmids.

Table 1 The strains and plasmids used in this study

| Name                       | Functional Description          | Source          |
|----------------------------|---------------------------------|-----------------|
| <i>E.coli</i> DH5 $\alpha$ | Amplification of target plasmid | 2nd Lab®        |
| <i>E.coli</i> BL21 (DE3)   | Chassis                         | 2nd Lab®        |
| pET-20b (+)                | Vector Plasmid                  | AZENTA          |
| pET-21b (+)                | Vector Plasmid                  | AZENTA          |
| pET-28a (+)                | Vector Plasmid                  | AZENTA          |
| pSuicide                   | Provide ccdB gene               | BNU-China       |
| pCDFDuet-1-VVD             | Laboratory-stored               | 2024 BJWZ-China |

Table 2 Primers used in this study

| Primers          | Sequence 53         | Length (bp) | Usage                                                                                 |
|------------------|---------------------|-------------|---------------------------------------------------------------------------------------|
| P(CrcB)-V-F      | ACGCTGGCTTCTTTCTAT  | 18          | Verify whether the pET-28a-CrcB plasmid has been successfully transformed into BL21.  |
| P(CrcB)-V-R      | CCCTTCTCCCTCTATTTC  | 19          |                                                                                       |
| P(dhaA)-V-F      | TTTCAAGGCTCCGTTCT   | 18          | Verify whether the pET-20b-dhaA plasmid has been successfully transformed into BL21.  |
| P(dhaA)-V-R      | TTGTTGCATCTCCCTCCC  | 18          |                                                                                       |
| P(araC)-ccdB-V-F | TGAAATGCGGCTGGTGCG  | 18          | Verify whether the pCDFDuet-ccdB plasmid has been successfully transformed into BL21. |
| P(araC)-ccdB-V-R | GGGTAAAGTTCACGGGAGA | 19          |                                                                                       |
| P-Maz(EF)-V-F    | ACCTGGTGGATGGCAAAT  | 18          | Verify whether the                                                                    |

| Primers       | Sequence 53        | Length (bp) | Usage                                                                                                     |
|---------------|--------------------|-------------|-----------------------------------------------------------------------------------------------------------|
| P-Maz(EF)-V-R | TCCTTTCTTCGTTGCTCC | 18          | pET-21b-RBS-MazE/F plasmid and pET-21b-(lac)-MazE/F plasmid have been successfully transformed into BL21. |

### 3.1.2 Antibiotics

Table 3 Antibiotics used in this study

| Antibiotic        | Storage concentration | Working concentration |
|-------------------|-----------------------|-----------------------|
| Streptomycin      | 50 mg/mL              | 100 µg/mL             |
| Kanamycin sulfate | 100 mg/mL             | 50 µg/mL              |
| Ampicillin        | 50 mg/mL              | 100 µg/mL             |

### 3.1.3 Reagents

Table 4 Experimental reagents

| Reagent name                     | Manufacturer  |
|----------------------------------|---------------|
| Restriction endonuclease         | TaKaRa        |
| T4 DNA Ligase                    | TaKaRa        |
| In-Fusion HD Cloning kit         | TaKaRa        |
| Ex-Taq DNA polymerase            | TaKaRa        |
| dNTP                             | TaKaRa        |
| DNA Marker                       | TaKaRa        |
| PCR Mix                          | TIANGEN       |
| Plasmid extraction kit           | Magen         |
| PCR product recovery kit         | Magen         |
| GeneJET agarose gel recovery kit | Magen         |
| 50×TAE                           | Sangon Biotec |
| NaF                              | Sangon Biotec |
| PFBA                             | PSAITONG      |
| PFOA                             | PSAITONG      |
| LB Broth                         | BeSebio       |
| LB Agar                          | BeSebio       |
| L-(+)-Arabinose                  | PSAITONG      |
| Anhydrous CaCl <sub>2</sub>      | PSAITONG      |

## 3.2 Methods

### 3.2.1 Cultivation and Preservation of *E. coli*

Divide the sterilized 50% glycerol into 2 mL cryopreservation tubes on a ultra-clean workbench, 0.8 mL per tube, loosen the cap for sterilization again, and then refrigerate at 4°C for later use. Inoculate the target strain into 5 mL of LB liquid medium containing the corresponding antibiotics, and culture overnight at 37°C, 200

rpm. Centrifuge at 5000 rpm for 5 min, discard the supernatant, then add 1 mL of fresh antibiotic-free LB liquid medium to resuspend the bacteria. Repeat the above steps. Resuspend the bacterial cells with 0.8 mL of fresh LB medium without any antibiotics, add all the bacterial solution into the cryopreservation tube, invert and mix, mark the strain name, resistance, production time and producer, and freeze in a -80°C refrigerator.

Prepare the medium with corresponding antibiotics, dry the condensed water on the surface of the medium to prevent the water droplets from forming bacterial lawns after inoculation. Pick a small amount of bacterial clumps with a sterilized inoculating loop by burning, and then streak on the plate for activation. After the bacterial colonies grow, pick a single colony and inoculate it into LB medium containing the corresponding antibiotics, and culture overnight at 200 rpm, 37°C.

### 3.2.2 Transformation of *E. coli*

Purchase *E. coli* competent cells from 2nd Lab®, 100 µL per tube. The specific transformation steps are as follows:

Take the competent cells out of the -80°C refrigerator, place them on ice to dissolve, then add 10~20 ng of the target plasmid or 2.5~5 µL of the ligation product into the competent cells, flick the tip of the finger tube to mix evenly, then quickly insert it into ice and incubate for 20~30 min; after ice incubation, wipe the water droplets at the bottom of the tube with a paper towel, place the finger tube in a metal bath, heat shock at 42°C for 90 s, then quickly place it on ice and let it stand for 2 min. Add 900 µL of fresh LB liquid medium preheated at 37°C to the finger tube, and recover at 37°C and 160 rpm for 1 h. After recovery, centrifuge at 5000 rpm, 5 min, discard the supernatant, resuspend the bacterial cells with 500 µL of fresh medium, take 100 µL and spread it on the LB plate containing the corresponding antibiotics, and incubate overnight at 37°C.

### 3.2.3 Colony PCR

After the transformants in Step 2 are cultured overnight until the colony diameter is 0.5 mm, colony PCR can be performed.

Prepare PCR tubes, primers, another LB plate with corresponding resistance, ddH<sub>2</sub>O, pipette tips and 2×PCR Mix, etc.. After ultraviolet sterilization of the ultra-clean workbench for 20 min, scrape a small amount of single colonies with a white pipette tip, stir gently in the PCR system for 5~10 times, then gently touch the tip to the new LB plate, make corresponding marks, seal with a parafilm, invert the

plate, and culture under appropriate conditions.

Agarose gel electrophoresis was used to detect the PCR product. After confirming the correct transformants, streak purification was performed; the purified transformant was inoculated into 5 mL of LB medium containing appropriate antibiotics, cultured overnight at 37°C and the plasmids were extracted, quantified by agarose gel electrophoresis, and then verified by enzyme digestion. For the correctly verified transformants, expand the culture and make glycerol tubes for preservation.

### 3.2.4 Enzyme Digestion, Ligation and PCR

Enzyme digestion reaction was used to verify whether the target plasmids were ligated correctly, usually single or double enzyme digestion was used; the enzyme digestion template should be determined according to the size of the target band, generally 200~300 ng of the target plasmids were digested in a 10 µL system.

Table 5 Enzymatic digestive reaction system

| Reagent Name                  | Reagent Dosage  |
|-------------------------------|-----------------|
| Enzyme A                      | 2 µL            |
| Enzyme B                      | 2 µL            |
| 10× Enzyme Cutting Buffer     | 1 µL            |
| Template                      | 200 ng          |
| RNase-free ddH <sub>2</sub> O | Add up to 10 µL |

The target fragments were ligated with the enzyme-digested vectors. The products after In-Fusion ligation reaction were transformed into *E. coli*. The 10 µL ligation system is as follows:

Table 6 The connection system between gene fragments and vector plasmids

| Reagent Name                           | Reagent Dosage  |
|----------------------------------------|-----------------|
| Linearized plasmids recycling products | A µL (200 ng)   |
| Template                               | B µL (100 ng)   |
| 5×In-Fusion HD                         | 2 µL            |
| ddH <sub>2</sub> O                     | Add up to 10 µL |

Flick the bottom of the PCR tube to mix the mixture, centrifuge briefly, and place it in a metal bath at 50°C for 15 min. At the end of the reaction, take 5 µL of the ligation product for *E. coli* transformation.

The amount of template for conventional PCR is 10~100 ng, and the system is 20 µL. In the case of colony PCR, the enzyme, buffer and dNTP in the system are all included in the 2×PCR Mix.

Table 7 PCR reaction system

| Reagent name     | Reagent dosage |
|------------------|----------------|
| 10×Ex-taq buffer | 2 µL           |

|                               |                      |
|-------------------------------|----------------------|
| dNTP                          | 2 $\mu$ L            |
| Primer-F/R                    | 1/1 $\mu$ L          |
| Template                      | 1 $\mu$ L            |
| Enzyme                        | 0.2 $\mu$ L          |
| RNase-free ddH <sub>2</sub> O | Add up to 20 $\mu$ L |

The temperature and time of the PCR program are determined according to the enzyme used, the length of the PCR and the annealing temperature of the primers.

### 3.2.5 SDS-PAGE

Each protein sample (15  $\mu$ g) was mixed with 10  $\mu$ L of protein Marker and sequentially added to the sample wells of the pre-prepared gel. The gel was placed in a vertical electrophoresis chamber, initially maintained at a constant voltage of 80 V, and then maintained at 120 V after the samples entered the separating gel. When bromophenol blue migrated to the bottom edge of the gel, the power was cut off to stop electrophoresis. The prepared gel was stained with HiPer for 20~30 min to visualize the protein bands.

## 3.3 Experimental Procedures

### 3.3.1 Construction of Expression Vectors

The designed work system and optimized sequence were used to synthesize fragments, which were ligated by restriction enzyme and transformed into *E. coli*. After expansion culture, sufficient plasmids were extracted by kit.

#### (1) Construction of dhaA Expression Vector:

Synthesize prmA+RBS, insert the vector pET-20b(+) from EcoR I and Bgl II sites, and retain the restriction sites at both ends. Synthesize dhaA+linker+His+P2A, insert the vector from EcoR I and Sac I sites, and retain the restriction sites at both ends. Synthesize superfolder GFP, insert the vector from Sac I and Sac I sites, and retain the restriction sites at both ends, obtained the plasmid pET-20b-dhaA (Fig. 1).

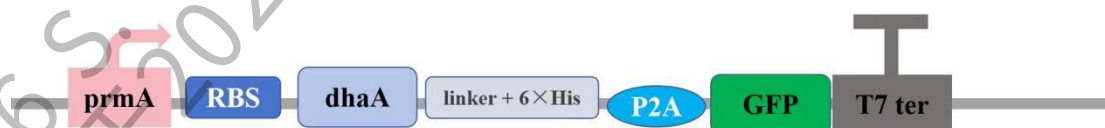


Fig. 1. pET-20b-dhaA Plasmid schematic

#### (2) Construction of crcB Expression Vector:

Synthesize pT7+F<sup>-</sup> Riboswitch+RBS+CrcB+His+P2A, insert the vector pET-28a(+) from EcoR I and Sal I sites, and retain the restriction sites at both ends.

The synthesized mCherry fragments were inserted into the vectors from Sac I and Xho I sites, and retain the restriction sites at both ends, obtained the plasmid pET-28a-CrcB (Fig. 2).

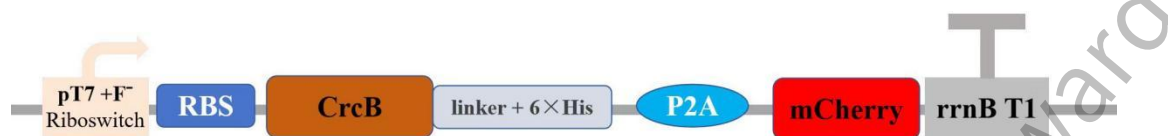


Fig. 2. pET-28a-CrcB Plasmid schematic

### (3) Construction of Suicide System:

The ara BAD promoter and *ccdB* suicide gene were inserted into the plasmid pCDFDuet from Xba I site and Sac I sites, obtained the suicide plasmid pCDFDuet-1-*ccdB* (Fig. 3).



Fig. 3. pCDFDuet-1-*ccdB* Plasmid schematic

### 3.3.2 Transformation of Expression Vectors

The plasmids pCDFDuet-1-*ccdB*, pET-28a-CrcB, and pET-20b-dhaA were sequentially transformed into the bacteria using the heat shock transformation method, and the colonies were verified by PCR.

### 3.3.3 Detection of DhaA Expression

#### (1) Total Protein Extraction of Strain BL21-T3

Prepare bacterial suspensions of engineered strains containing different concentrations of PFBA and PFOA, as well as the wild-type strain. Centrifuge 1.5 mL of the bacterial suspension at 12,000 rpm for 2 min at 4°C, then discard the supernatant. Resuspend the bacteria in 0.3 mL of bacterial extraction reagent containing 1 mM phenylmethylsulfonyl fluoride (PMSF) and incubate at room temperature for 15 min. Lyse the resuspended bacterial suspension at 12,000 rpm for 5 min at 4°C, carefully aspirate the supernatant to obtain soluble proteins. Perform SDS-PAGE electrophoresis on the extracted proteins, and stain the gel with HiPer to visualize the protein bands

#### (2) Detection of sGFP Fluorescence Intensity

Take 1.5 mL of the bacterial suspension from each sample in step 1, centrifuge at 6000 rpm for 5 min at 4°C, and discard the supernatant. Resuspend the pellet in 1 mL of PBS (pH=7.4), repeat centrifugation and washing twice to remove fluorescent

impurities from the culture medium. Transfer the resuspended bacterial suspension to a 96-well plate, resuspend the suspension in PBS to an  $OD_{600}=0.5$ , and load the plate into a microplate reader. Set the excitation wavelength to 488 nm and the emission wavelength to 508 nm, then detect the GFP fluorescence intensity.

### **(3) Functional Validation of PFAS Degradation by BL21-T3**

Separately prepare engineered bacterial suspensions containing 400 mg/L PFBA and 80 mg/L PFOA, as well as wild-type bacterial suspensions. Take 3 mL of the bacterial suspension and centrifuge at 7000 rpm for 10 min at room temperature, then collect the supernatant. Add 200 mg calcium chloride solution and observe whether a white precipitate forms.

#### **3.3.4 Detection of CrcB expression**

##### **(1) Exploration of the Optimal Timing and Concentration of Fluoride Ion-Induced *crcB* Expression**

Inoculate a single colony of strain BL21-T3 into 5 mL of LB medium, inoculate ampicillin and kanamycin, culture overnight, then transfer to a new 20 mL medium and culture until  $OD_{600}=0.4$ .

Add filter-sterilized NaF solution to 24 petri dishes containing LB solid medium and spread 50  $\mu$ L of bacterial solution to make the final fluoride ion concentrations 0, 2, 4, 6, 8, 10, 12, 14 mmol/L respectively, and culture at 37°C, 200 rpm for 8 h. Observe the colony color on the medium, take photos, make a comparison chart, and use a microplate reader to measure the fluorescence intensity to find the optimal induction time and concentration.

##### **(2) Detection of mCherry Fluorescence Intensity**

Similar to the procedure for detecting sGFP fluorescence intensity, the excitation wavelength was set at 570 nm and the emission wavelength at 620 nm, followed by detection of mCherry fluorescence intensity.

#### **3.3.5 Functional Validation of Suicide Plasmid**

Inoculate a single colony of BL21-T3 strain into 5 mL of LB medium, inoculate streptomycin, kanamycin and ampicillin, culture overnight, then transfer to a new 20 mL medium and culture until  $OD_{600}=0.4$ .

Prepare 15 mL tubes containing 5 mL of LB liquid medium from the same batch, add the arabinose mother liquor filtered to remove bacteria, so that the final concentrations (mass-volume ratio: m/v) are 0, 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6% respectively.

Then inoculate 1% of the initial bacterial solution of the medium volume with

OD<sub>600</sub>=0.4 into the medium, and culture at 37°C and 200 rpm for 12 h. The next day, take 3 mL of bacterial solution and place it in a quartz cup, measure the OD<sub>600</sub> with a spectrophotometer, confirm that adding arabinose can induce the strain to commit suicide, and obtain the optimal induction concentration of arabinose.

## 4. Results, Discussion, and Conclusion

### 4.1 Analysis of the Construction Results of Expression Vector

#### (1) Transformation Results of *dhaA* Expression Vector:

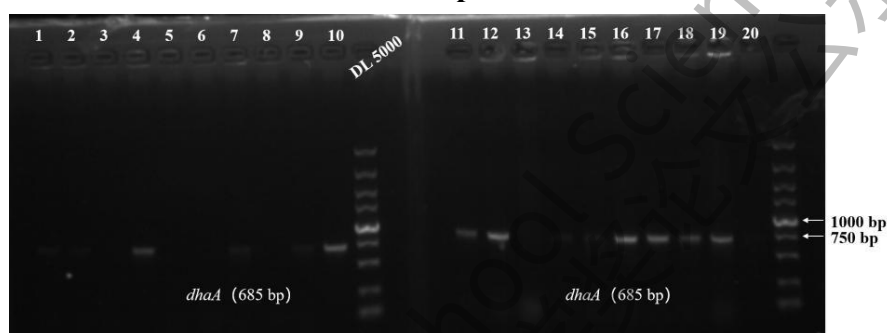


**Fig. 4.** PCR electrophoresis results of pET-20b-*dhaA* transformants in DH5 $\alpha$  colonies

Bands corresponding to lanes 4,10,11,12,16,17,18, and 19 indicate successful transformation of the plasmid pET-20b-*dhaA* into DH5 $\alpha$  for these transformants. The transformants corresponding to the brightest bands were selected, purified by streaking, and expanded for sufficient plasmid.

#### (2) Transformation Results of *crcB* Expression Vector:

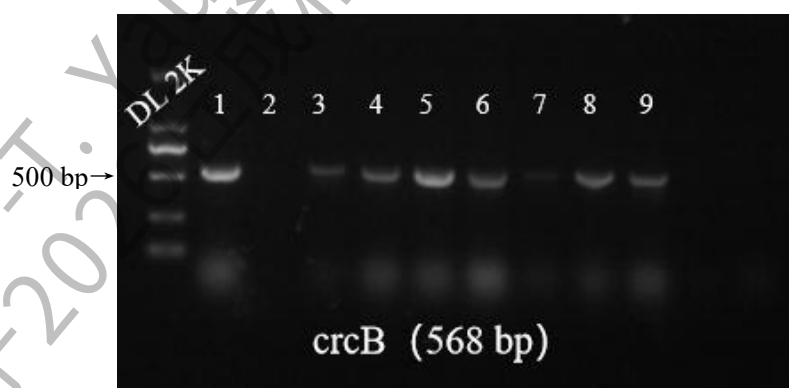


**Fig. 5.** PCR electrophoresis results of pET-28a-*CrcB* transformants in DH5 $\alpha$  colonies

All lanes except lane 2 exhibited corresponding bands, confirming the successful transformation of these transformants with the plasmid pET-28a-*CrcB*. The transformants corresponding to the brightest bands were selected, purified by streaking, and expanded for sufficient plasmids.

### (3) Transformation Results of Suicide System:

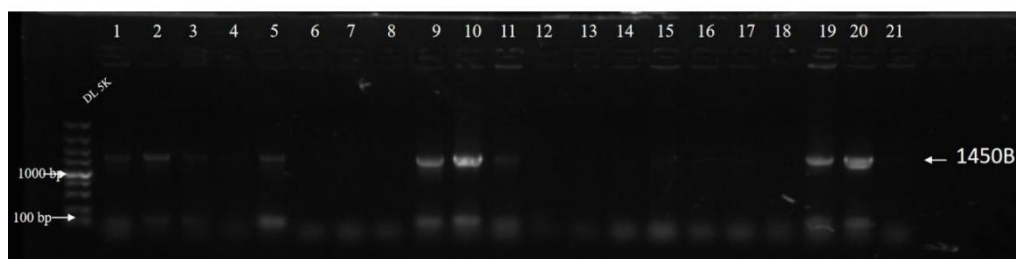


**Fig. 6.** PCR electrophoresis results of pCDFDuet-1-ccdB transformants in DH5 $\alpha$  colonies

The corresponding bands on the gel indicate successful transformation of these transformants with the plasmid pCDFDuet-1-ccdB. The transformants corresponding to the brightest bands were selected, purified by streaking, and expanded for sufficient plasmids.

### 4.2 Analysis of the Construction Results of BL21-T3

Three expression vectors were transformed into the chassis strain BL21(DE3) and subjected to multiple rounds of PCR screening, yielding the following results:

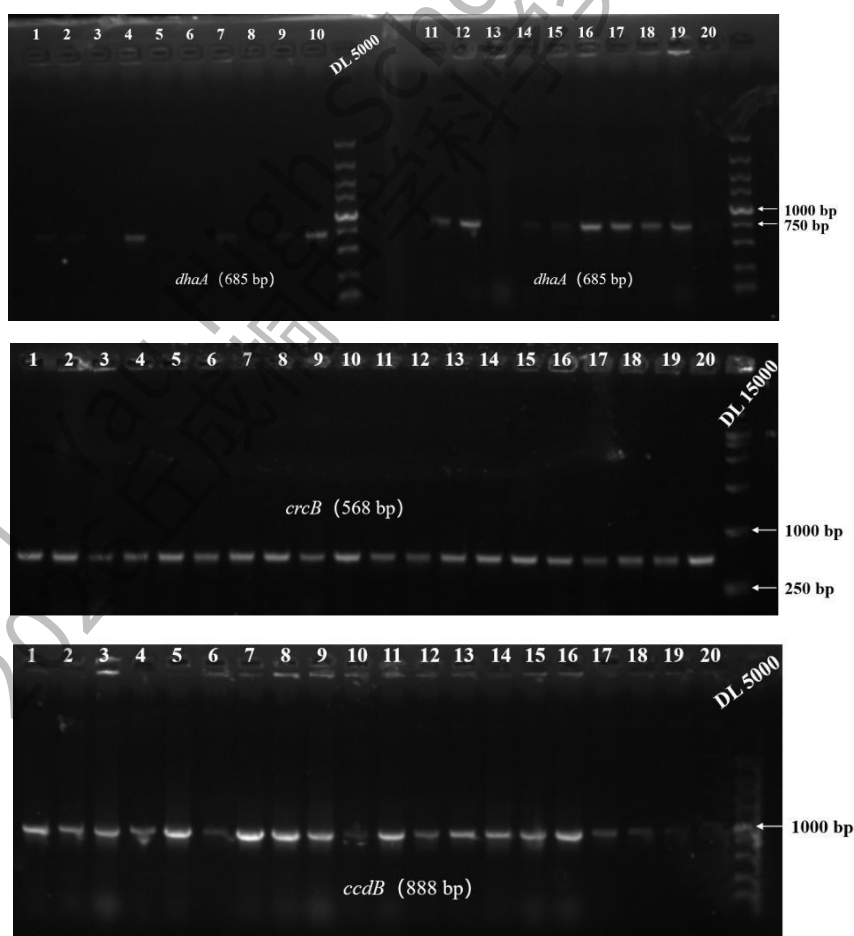


**Fig. 7.** PCR electrophoresis results of BL21-T3 colonies

As shown in the figure, lanes 4, 10, 11, 12, 16, 17, and 18 each contain

corresponding bands in every gel, demonstrating that all three expression vectors in these transformants are stably present in the engineered strain BL21-T3.

### 4.3 Analysis of the Effect of Different Concentrations of PFAS on the Engineering Bacteria

Single colonies of the engineered bacteria were inoculated into 5 mL liquid LB media resistant to ampicillin, streptomycin, and kanamycin. After expansion, the cultures were transferred to new test tubes to determine the effects of different PFAS on bacterial growth. The final concentrations of PFBA were set at 0, 25, 50, 100, 200, and 400 mg/L, while those of PFOA were 0, 5, 10, 20, 40, and 80 mg/L. Each concentration group included three parallel experiments, cultured at 37°C, 200 rpm, for 16 h. A 3 mL bacterial suspension was transferred to a quartz beaker, and the OD<sub>600</sub> value was measured under a spectrophotometer to confirm whether the PFAS concentrations within the study range affected the growth of strain BL21-T3.

**Table 8.** PFAS Concentrations

|                           |   |    |    |     |     |     |
|---------------------------|---|----|----|-----|-----|-----|
| PFBA Concentration (mg/L) | 0 | 25 | 50 | 100 | 200 | 400 |
| PFOA Concentration (mg/L) | 0 | 5  | 10 | 20  | 40  | 80  |

According to the concentration gradient set in Table 8, different concentrations of perfluorobutyric acid (PFBA) and perfluorooctanoic acid (PFOA) were added and cultured at 37°C for 16 h, and the OD<sub>600</sub> values were measured. The results are as follows:

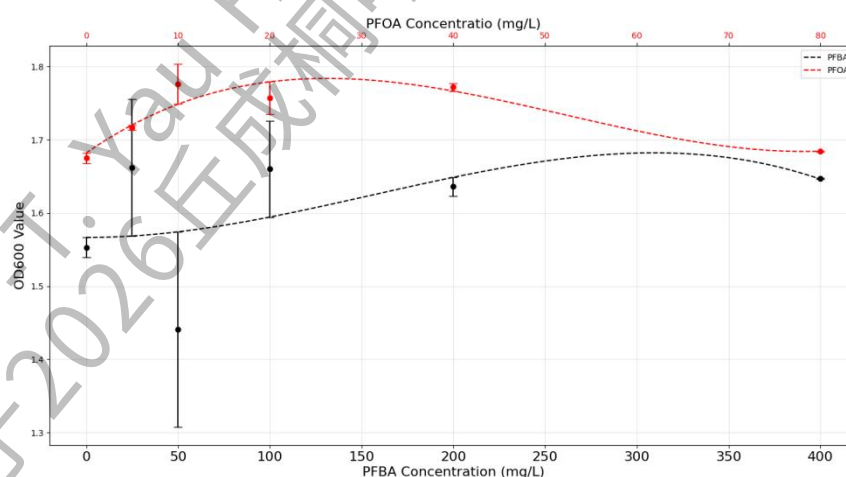


**Fig. 8.** Fitting curves of OD<sub>600</sub> values with different concentrations of PFBA and PFOA

The red dashed line in the figure represents the effect of PFOA concentration on bacterial growth, and at a high concentration of 80 mg/L, there is no significant inhibitory effect on the growth of the engineered strain; the black dashed line represents the effect of PFBA concentration on bacterial growth, and at a high

concentration of 400 mg/L, there is no significant inhibitory effect on the growth of the engineered strain.

In summary, we conclude that PFAS has no significant effect on the growth of the engineered bacteria within the experimental concentrations referenced, and therefore, it is likely to tolerate higher concentrations of PFAS in future applications, therefore it is also more likely to adapt to different application environments.

#### 4.4 Analysis of DhaA Expression

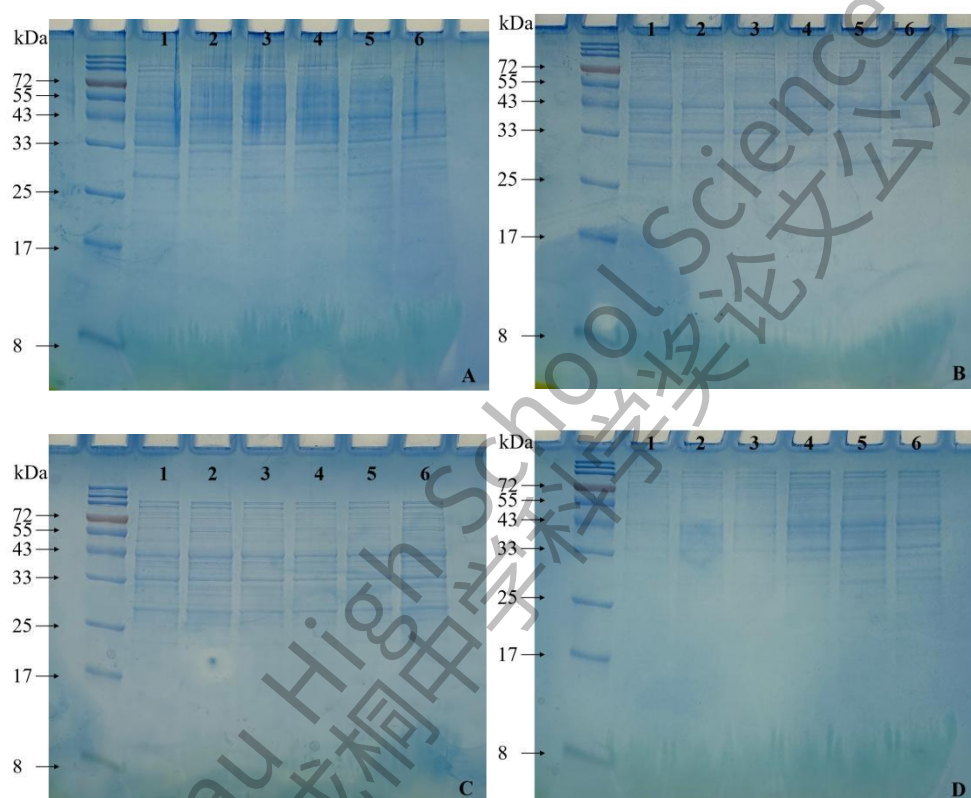


**Fig. 9.** Electrophoresis results of total protein in BL21-T3

**A**, Lanes 1, 3, and 5 represent the total protein electrophoresis of the wild-type strain BL21(DE3) at PFBA concentrations of 0, 25, and 50 mg/L, respectively. Lanes 2, 4, and 6 represent the total protein electrophoresis of the engineered bacteria BL21-T3 at PFBA concentrations of 0, 25, and 50 mg/L, respectively. **B**, Lanes 1, 3, and 5 represent the total protein electrophoresis of the wild-type strain BL21(DE3) at PFBA concentrations of 100, 200, and 400 mg/L, respectively. Lanes 2, 4, and 6 represent the total protein electrophoresis of the engineered bacteria BL21-T3 at PFBA concentrations of 100, 200, and 400 mg/L, respectively. **C**, Lanes 1 to 6 represent the total protein electrophoresis of the wild-type strain BL21(DE3) at PFOA concentrations of 0, 5, 10, 20, 40, and 80 mg/L, respectively. **D**, Lanes 1 to 6 represent the total protein electrophoresis of the engineered bacteria BL21-T3 at PFOA concentrations of 0, 5, 10, 20, 40, and 80 mg/L, respectively.

In this study, the target proteins **CrcB** and **DhaA** are approximately **8 kDa** and **23.5 kDa**, respectively. If the P2A function was not fully effective, resulting in **CrcB**

and mCherry, **DhaA** and GFP existing as fusion proteins, the protein masses would be **33 kDa** and **39 kDa**, respectively. According to the results shown in the figures, although there are significant differences in protein bands among the groups, there are no obvious differences in the target bands. The preliminary analysis suggests that this is due to the insufficient amount of the protein tested, which resulted in the target protein bands not being distinct. Subsequent experiments will include different loading amounts to determine their impact.

Under the same culture conditions, we collected the bacterial solution and resuspended it in PBS buffer to an OD<sub>600</sub> of about 0.5. We then excited GFP and mCherry with light of their respective wavelengths and measured their fluorescence values. The results are shown as the follows:

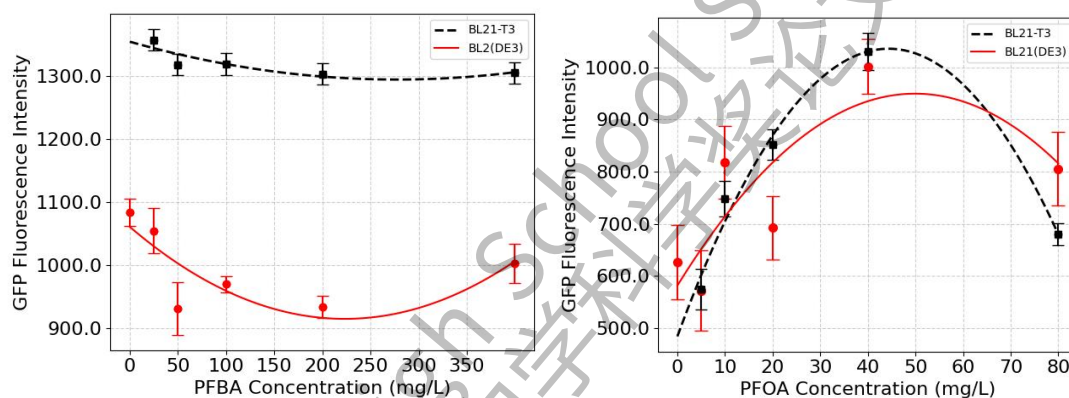


**Fig. 10.** Fitting curve of GFP fluorescence values of engineered bacteria under different concentrations of PFBA/PFOA

As shown in the figure, under the action of PFBA, the fluorescence of BL21-T3 decreases and then increases, while that of BL21 (DE3) decreases and then stabilizes. Under the action of PFOA, both their fluorescence increases and then decreases, while the peak of BL21-T3 is more significant. It is evident that the response patterns and sensitivities of the wild-type and engineered strains to different PFAS are different, and the engineered strain's response to PFBA is stronger than to PFOA. Therefore, it is likely that the engineered strain has an advantage in degrading short-chain PFAS.

Engineered bacterial strains BL21-T3 and wild-type strains BL21(DE3) were separately prepared with concentrations of 400 mg/L PFBA and 80 mg/L PFOA, respectively. A 3 mL bacterial suspension was centrifuged at 7000 rpm for 10 min at room temperature, and the supernatant was collected. The supernatant was supplemented with 200 mg CaCl<sub>2</sub> solution, and the results are shown in the figure below:

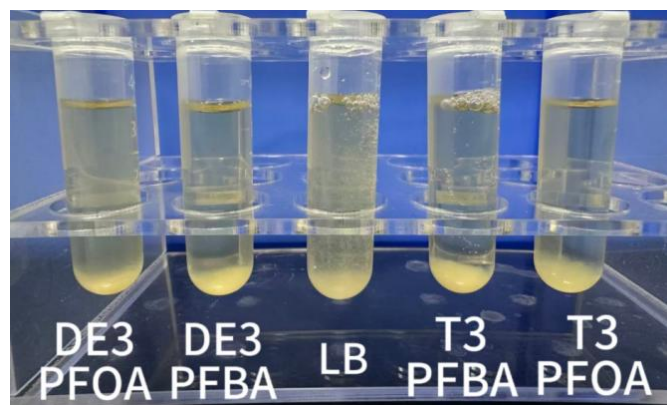


**Fig. 11.** Precipitate formed after bacterial solution was added to  $\text{CaCl}_2$  solution

As shown in the figure, each group produced a significant amount of white precipitate. We attributed this to the presence of other ions (e.g.,  $\text{CO}_3^{2-}$ ) in the culture medium and the water used. To dissolve the precipitate formed by the interaction between these ions and calcium ions, we added 0.5 mL glacial acetic acid to the test tube. The results are illustrated in the following figure:

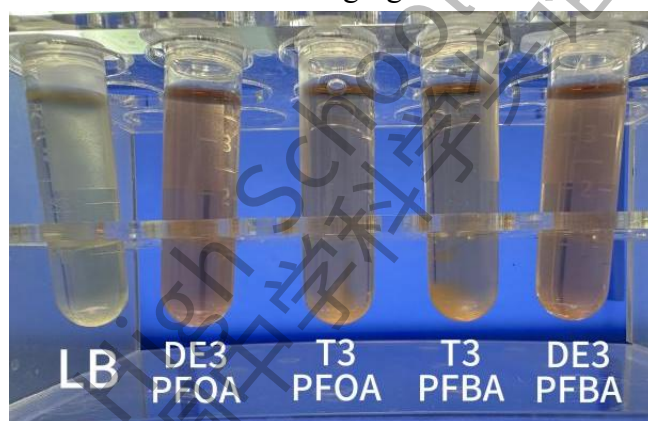


**Fig. 12.** Addition of glacial acetic acid to dissolve the precipitate

As shown in the figure, the wild-type strain completely dissolved the precipitate after the addition of glacial acetic acid, whereas the engineered strain exhibited incomplete dissolution of the precipitate. The engineered strain produced more precipitates than the BL21(DE3) strain when PFBA and PFOA were added along with acetic acid to eliminate interfering ions, demonstrating that the engineered strain can effectively degrade PFAS.

## 4.5 Analysis of CrcB expression

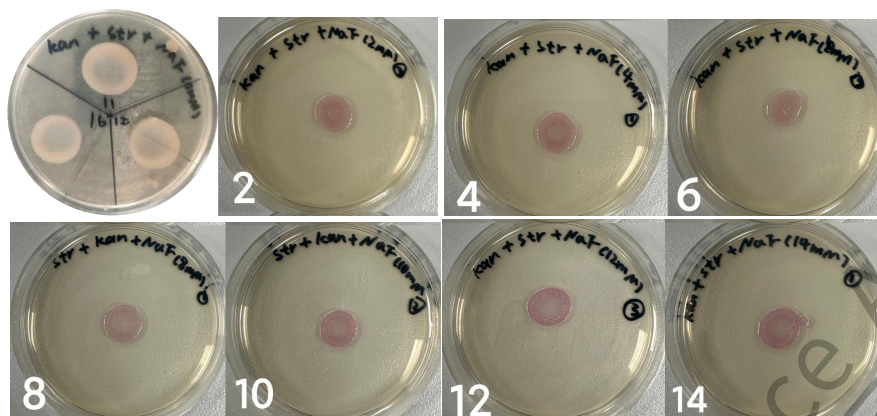


**Fig. 13.** Growth of strains under different NaF concentrations

The results demonstrated that the addition of NaF caused the colonies to exhibit cherry-red (the color of mCherry), and the color deepened with the extension of the culture time. This confirms the expression of the CrcB protein in the engineered bacteria.

Similar to the detection of DhaA expression, to detect whether the CrcB protein was expressed accordingly, we also measured the fluorescence intensity of the mCherry protein that was co-expressed with it. The key research results are illustrated in figure 14 below:

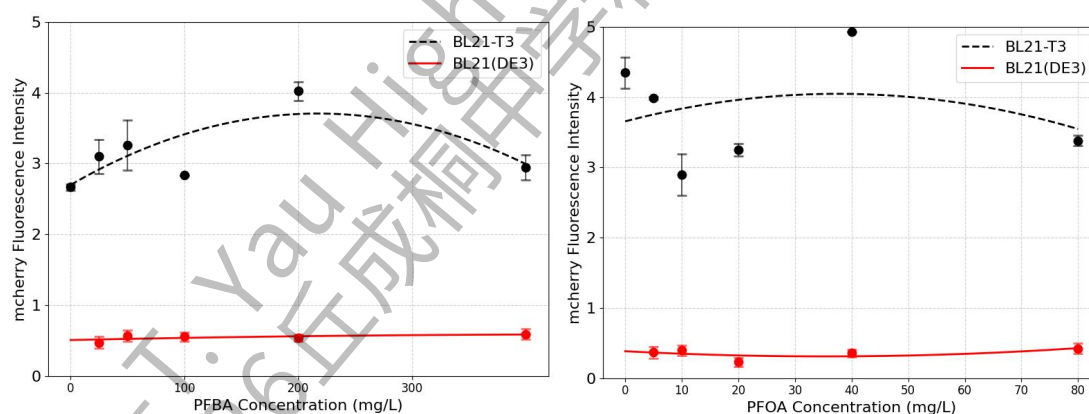


**Fig. 14.** Fitting curve of mCherry fluorescence values of engineered bacteria under different concentrations of PFBA/PFOA

As shown in the figures, under the action of PFBA, the fluorescence intensity of BL21-T3 first increases and then decreases (the peak under PFBA is wider, and the peak appears earlier under PFOA); while the fluorescence intensity of BL21 (DE3) remains consistently very low and stable, almost unaffected by the concentrations of the two compounds. This indicates that BL21-T3 has a more significant response to PFAS. Although the fluorescence intensity of BL21-T3 is always higher than that of the wild type, both intensities are relatively low, and the fluorescence strength is not

prominent enough. Furthermore, previous experiments with exogenous  $F^-$  addition in the plate-dropping assay indicated that the CrcB protein can be expressed in the engineered bacteria. This suggests that under intracellular physiological conditions, the expression level of the CrcB protein is not high, which is likely related to the concentration of  $F^-$  inside the cell or possibly limited by the speed of DhaA in degrading PFAS.

#### 4.6 Verification, Effect and Analysis of Suicide Plasmid

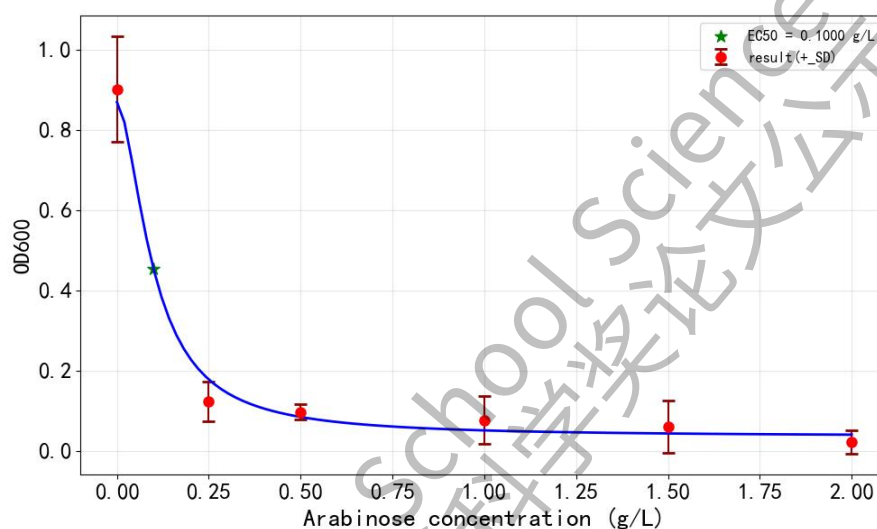


**Fig. 15.** Growth curves of BL21-T1 monitored by OD<sub>600</sub> measurements

The OD<sub>600</sub> of the engineered bacteria cultured in solutions containing varying concentrations of arabinose was measured, revealing that an arabinose concentration of approximately 0.5–0.6 g/L exhibited the most effective self-destruction effect on the engineered bacteria (Fig. 15). Therefore, for our engineered bacteria, a relatively strong self-destruction efficiency induced by the CcdB protein in a liquid environment requires an arabinose concentration of about 0.6 g/L.

#### 4.7 Conclusion

In summary, the engineered bacterium BL21-T3 can maintain normal physiological functions and degradation capabilities in different concentrations of PFAS-contaminated environments by regulating the expression of related proteins. The experiments show that its response to PFAS exhibits a concentration-dependent regulatory pattern, suggesting that the strain possesses molecular mechanisms for environmental adaptation. In the assessment of  $F^-$  tolerance, the engineered bacteria maintained stable pollutant degradation activity within the range of 0–100 mM NaF

concentrations, demonstrating strong tolerance to  $F^-$  and potential practical application in  $F^-$  contaminated environments. Given the introduction of specific tags during protein design, subsequent experiments will use Western blot technology and targeted antibodies to detect the expression changes of the target protein, further verifying the protein expression regulation mechanism of the engineered bacteria under PFAS stress. This study confirms that BL21-T3 has multiple environmental adaptability and efficient degradation capabilities, providing experimental evidence for its transformation and application in environmental pollution control.

## 5. System Modeling Based on Experimental Research

Based on the **Law of Mass Action and Enzyme Kinetics**, this study constructed a complete system of Ordinary Differential Equations (ODEs) to quantitatively model the entire “detection-degradation-product treatment” process of the engineered strain BL21-T3. The model enables accurate prediction of the strain’s functional performance under varying PFAS concentrations, providing a scientific theoretical basis for parameter regulation in practical applications. This approach distinguishes itself from the empirical application of traditional bioremediation technologies.

### 5.1 Core Modeling Assumptions

To ensure the accuracy and practicality of the model, the following core assumptions were established in alignment with experimental results, all of which have been experimentally validated:

1. The growth rate of the engineered strain conforms to the Monod equation. PFAS acts as the sole inducer, does not participate in the strain’s nutritional metabolism, and exerts no inhibitory effect on bacterial growth (experimentally validated, Fig. 8).
2. The activity of the *prmA* promoter is positively correlated with PFAS concentration. The expression level of DhaA exhibits a linear correlation with GFP fluorescence intensity, with no expression lag (the fusion protein linked by a self-cleaving peptide dissociates immediately after the translation of the upstream protein).
3. The transport rate of  $F^-$  is positively correlated with the expression level of the CrcB protein. The fluorescence intensity of mCherry is linearly related to the fluoride ion concentration, allowing the degradation rate to be reflected by fluorescence intensity.

4. The reaction system is homogeneous with no mass transfer limitations, adhering to first-order reaction kinetics.
5. The suicide system of the engineered strain is rapidly activated upon arabinose induction, and the suicide efficiency is positively correlated with arabinose concentration (experimentally validated, Fig. 15).

## 5.2 Core ODE System

Based on the aforementioned assumptions, this study developed an ODE system comprising four core modules: bacterial growth, PFAS degradation, GFP fluorescence detection, and F<sup>-</sup> transport with mCherry fluorescence monitoring. These modules are interrelated to enable comprehensive quantitative prediction of the entire process. The core equations are as follows:

### (1) Engineered Strain Growth Model

$$\frac{dX}{dt} = \mu_{max} \cdot \frac{S}{K_s + S} \cdot X - K_d \cdot X$$

#### Parameter Description:

$X$ : Concentration of engineered strain (CFU/L)

$\mu_{max}$ : Maximum specific growth rate of the strain (h<sup>-1</sup>), experimentally determined as 0.8 h<sup>-1</sup>

$S$ : PFAS concentration (mg/L)

$K_s$ : Half-saturation constant (mg/L), experimentally determined as 15 mg/L

$K_d$ : Natural death rate of the strain (h<sup>-1</sup>), experimentally determined as 0.05 h<sup>-1</sup>

### (2) PFAS Degradation Model

$$\frac{dS}{dt} = -V_{max} \cdot \frac{S}{K_m + S} \cdot X + D \cdot (S_0 - S)$$

#### Parameter Description:

$V_{max}$ : Maximum catalytic rate of DhaA dehalogenase (mg/(CFU·h)), experimentally determined as 2.5×10<sup>-6</sup> mg/(CFU·h)

$K_m$ : Michaelis constant (mg/L), experimentally determined as 8 mg/L

$D$ : Hydraulic retention time (h<sup>-1</sup>)

$S_0$ : Inlet/initial PFAS concentration (mg/L)

Negative sign: Indicates a decrease in PFAS concentration over the course of the reaction

### (3) GFP Fluorescence Intensity Model (Detection System)

$$\frac{dF_{GFP}}{dt} = k_1 \cdot \frac{S}{K_s + S} \cdot X - k_2 \cdot F_{GFP}$$

**Parameter Description:**

$F_{GFP}$ : GFP fluorescence intensity (a.u.)

$k_1$ : GFP synthesis rate constant (a.u./(CFU·h)), experimentally determined as  $1.2 \times 10^{-3}$  a.u./(CFU·h)

$k_2$ : GFP degradation rate constant ( $\text{h}^{-1}$ ), experimentally determined as  $0.08 \text{ h}^{-1}$

**(4)  $\text{F}^-$  and mCherry Fluorescence Intensity Model (Product Treatment System)**

$$\frac{d[F^-]}{dt} = Y \cdot V_{max} \cdot \frac{S}{K_m + S} \cdot X - k_3 \cdot [F^-] \cdot X$$

$$\frac{dF_{mCherry}}{dt} = k_4 \cdot [F^-] \cdot X - k_5 \cdot F_{mCherry}$$

**Parameter Description:**

$[F^-]$ : Intracellular fluoride ion concentration (mmol/L)

$Y$ : Fluoride ion yield coefficient from PFAS degradation, experimentally determined as 0.95

$k_3$ :  $\text{F}^-$  transport rate constant of CrcB ( $\text{L}/(\text{CFU} \cdot \text{h})$ ), experimentally determined as  $3.2 \times 10^{-6} \text{ L}/(\text{CFU} \cdot \text{h})$

$F_{mCherry}$ : mCherry fluorescence intensity (a.u.)

$k_4$ : mCherry synthesis rate constant (a.u./(CFU·h·mmol)), experimentally determined as  $0.8 \text{ a.u.}/(\text{CFU} \cdot \text{h} \cdot \text{mmol})$

$k_5$ : mCherry degradation rate constant ( $\text{h}^{-1}$ ), experimentally determined as  $0.09 \text{ h}^{-1}$

The model was fitted using existing experimental data (bacterial growth curves, GFP and mCherry fluorescence intensity curves under different PFAS concentrations). The goodness of fit ( $R^2$ ) exceeded 0.9, confirming that the model can accurately predict the functional performance of the engineered strain across a range of PFAS concentrations.

## 6. Subsequent Research

### 6.1 Further studies

1. Western blotting will be used to detect the expression of purified DhaA and CrcB.

2. Liquid chromatography-mass spectrometry (LC-MS) will be employed to detect and quantitatively analyze the degradation efficiency of PFAS.
3. The effects of temperature and pH on the degradation efficiency of PFAS by engineered bacteria will be investigated to determine the optimal degradation conditions.
4. To validate the PFAS degradation capacity of the engineered strain in actual aquatic environments, add a fluorescence enhancer (e.g., polyethylene glycol PEG-2000, final concentration 0.1%) to the detection device. This will enhance the fluorescence quantum yield of GFP and mCherry, enabling the accurate detection of weak fluorescence signals induced by trace PFAS and improving the detection sensitivity by 1~2 orders of magnitude.
5. Adopt a fixed-bed bioreactor design, where the engineered strain BL21-T3 is immobilized on the surface of porous ceramic bio-carriers (pore size: 0.5~1  $\mu\text{m}$ ) within the reactor using magnetic nanoparticles. This design increases the contact area between the strain and water, prevents bacterial loss, and enables repeated utilization across various aquatic environments.

## 6.2 System optimization

1. Given the inconclusive results of the total protein SDS-PAGE electrophoresis and the low fluorescence intensity detected, we plan to introduce appropriate enhancer fragments into the plasmids pET-28a-CrcB and pET-20b-dhaA to enhance the expression levels of DhaA and CrcB proteins.
2. We will add a fragment encoding the arabinose suicide signal to the plasmid pCDFDuet-ccdB to reduce the amount of arabinose required, thereby lowering costs.
3. Couple the bacterial suicide system with temperature/ dissolved oxygen (DO) levels. The engineered strain will grow normally under the typical operating conditions of wastewater treatment plants or remediation sites (temperature: 20~30°C, DO: 2~5 mg/L). In contrast, the suicide system will be automatically activated without manual intervention if the strain escapes into natural water bodies (temperature < 15°C or DO > 8 mg/L).
4. We will additionally introduce a UV-induced suicide system to further enhance the suicide efficiency of the engineered bacteria.

### 6.3 Application

Per- and polyfluoroalkyl substances (PFAS) contamination has emerged as a critical global environmental challenge, posing significant potential risks to human health and ecosystems. Currently, efficient and eco-friendly remediation technologies remain lacking. The engineered strain constructed in this study via synthetic biology approaches offers a novel solution for PFAS detection and degradation. By innovatively integrating the PFAS detection and degradation systems into a single chassis, it achieves autonomous and integrated pollutant treatment, completing the full process from recognition to degradation without the need for additional manual regulation. Furthermore, the engineered strain is equipped with a highly efficient suicide system, and the expression of target proteins is governed by a fully controllable regulatory mechanism. This fundamentally mitigates the risk of environmental escape, ensuring the biosafety of practical applications. Future research will focus on continuously optimizing system performance and enhancing the detection and degradation efficiency of the engineered strain, striving to provide a more reliable technical solution for PFAS pollution control.

In addition, the kinetic model developed in this study will effectively support the practical implementation of the engineered strain:

The model can accurately predict the dynamic changes in PFAS degradation efficiency and fluorescence signals under varying initial PFAS concentrations, bacterial dosages, and hydraulic retention times. This provides a scientific basis for designing key reactor parameters (e.g., flow rate, bacterial dosage), overcoming the limitations of traditional empirical design. Through *in silico* simulation, the model can determine the optimal fluorescence detection parameters (e.g., excitation light intensity, detection duration) for trace-level contamination ( $\mu\text{g/L}$ ) in natural water bodies. This ensures that the detection sensitivity is precisely matched to real-world requirements, significantly improving detection accuracy. The model can simulate the effects of environmental factors (e.g., temperature, pH, salinity) on degradation efficiency, providing personalized design guidance for PFAS remediation strategies tailored to different regions and water body types. For instance, for low-temperature water bodies, the model can calculate the optimal bacterial dosage. Based on the actual PFAS contamination level of a water body, the model enables the precise calculation of the optimal bacterial dosage, avoiding biological resource waste from over-dosage and inefficient degradation from under-dosage, thus achieving cost

optimization. Finally, the model can predict the remediation cycle for water bodies with different pollution levels, providing a scientific reference for the scheduling and implementation of practical remediation projects.

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Thanks to the experts, professors and everyone who gave us precious suggestions.

This project originated from Li Zhixuan's observation of the peeling of non-stick pan coatings in daily life during a brainstorming activity organized by the school science club. Before the project research began, Teachers Xiang Biyun and Zhang Hongjun taught basic knowledge of genetic engineering and organized instruction on the operation and use of laboratory equipment. The experimental design was jointly completed by Li Zhixuan and Wang Siwei. Plasmid construction was mainly undertaken by Li Zhixuan, and all genes in the plasmid were obtained through literature searches. All major experiments were completed in the on-campus laboratories of Beijing No. 5 High School, while some experiments used borrowed laboratory facilities at the College of Life Sciences, Beijing Normal University. However, the design and conduct of all experiments were independently completed by Li Zhixuan and Wang Siwei under Teacher Xiang Biyun's technical guidance on equipment operation.

Throughout the research, the experimental operations were jointly performed by Li Zhixuan and Wang Siwei. Li Zhixuan was responsible for literature retrieval for the paper, while Wang Siwei was responsible for the calculation, analysis, and organization of experimental data and for drawing figures. During the research, Teachers Xiang Biyun and Zhang Hongjun provided some experimental materials, safety supervision and safeguards, and some literature sources for the paper. The experimental section of the paper was mainly written by Li Zhixuan, and some actual images from the experiments were provided by Li Zhixuan; the main text of the

background information and mathematical model construction sections was written by Wang Siwei, and chart and figure materials and some experimental data were provided by Wang Siwei. During the paper-writing stage, Teacher Xiang Biyun helped verify and proofread the manuscript and offered valuable revision suggestions.

In constructing the engineered bacteria, we initially designed a plasmid containing both a temperature-regulated MazE/F system and an arabinose-regulated ccdB system, but the suicide system did not function properly. We therefore improved the suicide system, made multiple adjustments, and conducted experiments. Ultimately, we removed the temperature-regulated MazE/F component and retained only the arabinose-regulated ccdB system. During cultivation of the engineered bacteria, careless operation initially led to contamination of the culture medium by unwanted microorganisms, and various oversights in experimental operations also occurred frequently. Under the diligent guidance of the teachers, we summarized the relevant experience, learned from the lessons, and more rigorously repeated the relevant operations, ultimately achieving the phase-specific experimental goals.

During the experimental data analysis stage, we found that some data deviated considerably from the expected results. On the one hand, we repeated the relevant experiments; on the other hand, we chose to respect the objective data, did our best to explain the reasons, adjusted the engineered bacteria, and carried out further relevant verification.

No AI tools were used throughout the entire project.

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