

Many birds with one stone: When bioremediation meets synthetic biology

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Organic pollutants emerged as a significant health hazard to humans with far-reaching consequences for their toxicity, carcinogenicity, mutagenicity, and environmental persistence. While most of the pollutants can be degraded or transformed by certain microorganisms, real-world polluted sites, like sea areas polluted by oil refinery wastewater, often contain multiple pollutants, inhibiting bioremediation using a single microbial strain.^{1,2} Since 2021, researchers from Shanghai Jiao Tong University, Shenzhen Institute of Advanced Technology, Chinese Academy of Sciences, and Chinese Academy of Agricultural Sciences have reported the construction of an engineered *Vibrio natriegens* strain to remediate five organic pollutants in industrial wastewater and soil. The 4-year project has resulted in significant progress and was published on May 7, 2025.³ Here is a short summary of the major achievements in this project (Figure 1).

The salinity in industrial wastewater varies widely and demands robust bioremediation strains with high salinity tolerance. *V. natriegens*, an emerging synthetic chassis cell, demonstrated rapid growth in saline media and industrial wastewater samples. Further study revealed *V. natriegens*' resistance to mono-/polycyclic pollutants, making it the optimal chassis among *P. putida*, *B. xenovorans*, and other candidate bacterial strains. To enhance

genome editing, *V. natriegens* strain, Vmax, was integrated with *Vibrio cholerae*'s natural transformation global regulatory factor *VchtfX*, which was under the control of an inducible promoter (P_{TT}), boosting genome integration efficiency at neutral genomic sites from 1% to 78%. This developed the strain Vmax into the superior synthetic biology chassis VCOD-2, enabling fast and high-efficiency genome editing under mild conditions.

The sequences of degradation gene clusters were optimized for expression in VCOD-2: the over-lapped genes were separated, and non-coding sequences between genes were removed and substituted by artificial ribosome binding site (RBS) sequences, then inducible promoters and terminators were added to the start and end of the gene clusters. To reduce the cost of synthesizing long gene clusters, the degradation gene clusters exceeding 5 kbp were first synthesized in fragments of approximately 3 kbp, then assembled *in vivo* using *S. cerevisiae*. To develop a complex organic pollutant remediation strain, the complete series of degradation gene clusters were inserted into the genome of VCOD-2 using an iterative editing method INTIMATE. This method is used to insert long DNA fragments in the form of multiple 10-20 kbp DNA fragments with only 2 resistance markers, thereby improving the maximum allowable length of inserted DNA. The final engineering strain,

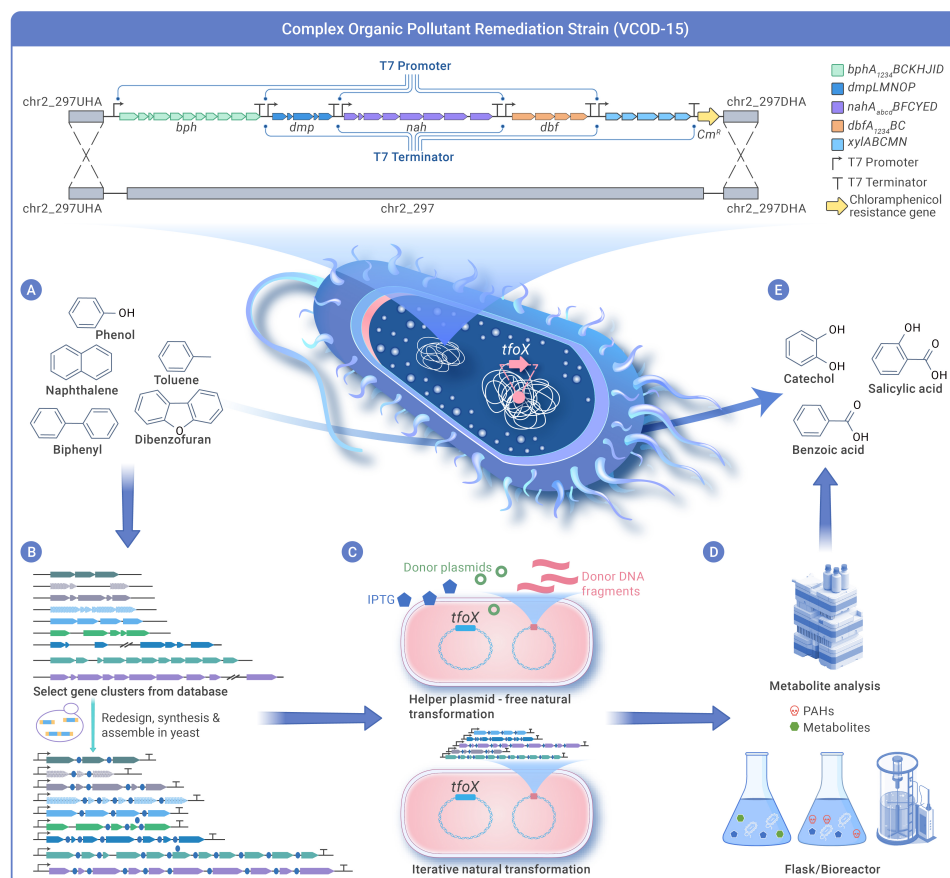


Figure 1. Schematic for development of complex organic pollutant remediation strain (VCOD-15) construction

A complex pollutant remediation strain was developed using the 'design-building-testing-learning' strategy. (A) Monoaromatic hydrocarbons (e.g. toluene and phenol) and polycyclic aromatic hydrocarbons (e.g., biphenyl, naphthalene and dibenzofuran) were chosen as the target organic pollutants to be degraded. (B) During the design stage, the degradation gene clusters were redesigned, synthesized, and assembled in yeast. (C) During the building stage, the gene clusters were inserted into the genome of the chassis cells. The *tfoX* gene from *V. cholerae* was inserted into the *V. natriegens* Vmax genome to amplify its natural transformation efficiency. An iterative natural transformation method known as INTIMATE was developed to insert all gene clusters into the Vmax genome. (D) During the testing stage, the degradation strains were exposed to saline media and industrial wastewater containing pollutants and were tested to monitor the conversion of pollutants into harmless substances. (E) During the learning stage, functional gene clusters characterized as effective when testing the various strains were pooled and inserted into a single chassis cell to generate a complex organic pollutant remediation strain.

VCOD-15, was capable of remedying 90% of all five pollutants in industrial wastewater and saline-contaminated soil. Stable isotope tracing (using ¹³C-labeled biphenyl, phenol, and ²H-labeled naphthalene, dibenzofuran, and toluene) confirmed intermediates aligned with designed pathways.

VCOD-15 exemplifies nature-inspired synthetic biology, offering a scalable solution for complex organic pollutants in environments. As synthetic biology advances, such engineered strains promise to bridge the gap between laboratory innovation and global environmental challenges. The upgrade and improvement of VCOD-15 is currently underway, including but not restricted to incorporating biocontainment systems and pollutant-responsive biosensor systems.⁴ This progress will surely pave the way to more intelligent and safer complex organic pollutant remediation strains.

With continuous advancement in improving the strategy in redesigning the degradation gene clusters and utilization of other chassis strains, researchers anticipate the construction of strains that can remediate more pollutants in multiple environments. The researchers developing VCOD-15 are currently focusing on the governance challenges posed by emerging pollutants, specifically referring to chemical substances that have been recently identified or have attracted attention, posing potential threats to the ecological environment and human health, but for which effective control systems have not yet been established. These substances include perfluorooctane sulphonate (PFOS), perfluorooctanoic acid and its salt-related compounds (PFOAs), and nonylphenol as typical representatives. The distribution range and concentration levels of these substances in the environment are not fully understood, but even in trace amounts, their persistent bioaccumulation characteristics may pose significant ecological risks. Compared to pollutants such as polycyclic aromatic hydrocarbons, which can be generated through natural processes (such as forest fires and volcanic activity), perfluorinated compounds (especially PFOAs), as chemical products synthesized artificially in the mid-20th century, are difficult to degrade through conventional enzymatic reactions due to the high chemical stability of their C-F bonds in natural environments.⁵ Moreover, existing research still has significant gaps in understanding the related degradation of metabolic genes and functional enzymes. To overcome this bottleneck, the researchers propose utilizing chromosome 2 of VCOD-15 (with a smaller genome size, higher tendency for

horizontal gene transfer, and mutation rate) as a genetic engineering vector. By directing the implantation of known dehalogenation genes and implementing adaptive laboratory evolution (ALE) strategies, the team aims to accelerate the construction of efficient perfluorinated compound-degrading bacterial strains and optimize related metabolic pathways.

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DECLARATION OF INTERESTS

Junbiao Dai is an Editorial Board member of *The Innovation Life*. He was blinded from reviewing or making final decisions on the manuscript. The article was subject to the journal's standard procedures, with peer review handled independently of this Editorial Board member and their research groups. The other authors declare that they have no conflicts of interest.