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# SYNTHETIC BIOLOGY ENGINEERING OF BACTERIAL SYSTEMS FOR ATMOSPHERIC METHANE REDUCTION APPLICATIONS

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## ABSTRACT

**Background:** Atmospheric methane is a major driver of global warming due to its high capacity to trap heat and its increasing emissions from industrial and agricultural activities. Synthetic biology provides new ways to engineer methanotrophic bacteria for efficient capture and conversion of methane under atmospheric conditions.
**Objective:** To develop bacterial systems with enhanced methane oxidation efficiency and environmental adaptability for mitigating atmospheric methane.
**Methodology:** Methanotrophic bacterial strains were genetically engineered by means of synthetic biology approaches, e.g. methane monooxygenase overexpression, optimization of carbon assimilation pathways and stress-resistance modules. Engineered strains were grown under low methane concentrations (1.8-5 ppm) and methane consumption was determined by gas chromatography . Biomass productivity and metabolic stability were also examined under simulated environmental stress conditions.
**Findings:** Methane uptake rate was increased by 2.5-fold in engineered strains (31.4 µmol/h vs. 12.5 µmol/h in wild-type strains). Furthermore, increased biomass production and enhanced oxidative stress tolerance were observed, reflecting stable metabolic performance under atmospheric methane conditions.
**Conclusion:** Methanotrophic bacteria with synthetic biology engineering have great promise for sustainable and scalable systems for mitigation of atmospheric methane and environmental biotechnological applications.

**KEYWORDS:** Synthetic biology, methanotrophs, atmospheric methane, methane oxidation, metabolic engineering, greenhouse gas mitigation, bacterial engineering, bioremediation.

## 1 INTRODUCTION

### 1.1 Climate Change and Methane Emissions

Climate change is one of the most serious environmental challenges that affect ecosystems, biodiversity and human society around the globe. Greenhouse gases (GHGs) such as carbon dioxide (CO2), methane (CH4), nitrous oxide (N2O), and fluorinated gases are important for global warming as they trap heat in the Earth's atmosphere [1]. Among these gases, methane is considered to be particularly dangerous, with a global warming potential that is about 28-34 times that of carbon dioxide over a 100 year period [2]. The anthropogenic activities have increased the atmospheric methane concentration rapidly. Methane mitigation is an important priority for climate stabilization [3]

Agriculture, landfills, fossil fuel extraction and natural wetlands are the main sources of methane emissions. Agricultural activities like livestock farming and rice cultivation generate significant methane through enteric fermentation and anaerobic decomposition [4]. Landfills are a source of methane production due to anaerobic microbial degradation of organic waste [5]. Methane leakage from oil, natural gas and coal extraction also accounts for a significant fraction of atmospheric emissions [6]. Wetlands are also a significant natural source of methane from microbial methanogenesis under oxygen-limited environments [7]. Methane accumulation in the atmosphere contributes to global warming and accelerates climate-related disasters.

### 1.2 Need for Biological Methane Mitigation

Conventional methods for methane mitigation (e.g., thermal oxidation, catalytic combustion, physical adsorption) are usually energy intensive and operationally expensive [8]. These technologies may also be inefficient at low concentrations of atmospheric methane . Microbial methanotrophs that can oxidize methane to carbon dioxide and biomass under ambient conditions provide a sustainable alternative to biological methane mitigation [9].

Methanotrophs oxidize methane as their primary carbon and energy source via methane monooxygenase (MMO) dependent pathways. Microbial methane oxidation is environmentally friendly, energy-efficient and adaptable to diverse ecosystems compared with physicochemical approaches [10].

### 1.3 Synthetic Biology in Environmental Engineering

Recent advances in synthetic biology have allowed engineering of bacterial systems with improved methane utilization properties. CRISPR-Cas genome editing technologies can precisely edit genes involved in methane oxidation pathways and stress tolerance [11]. Optimization of metabolic pathways can enhance carbon assimilation efficiency and biomass production in engineered methanotrophs. Synthetic gene circuits can also enable dynamic control of methane-responsive metabolic functions under different environmental conditions [12]. These innovations provide new opportunities for the development of efficient bacterial systems for the mitigation of atmospheric methane and for sustainable biotechnological applications.

### 1.4 Aim and Objectives

**Aim**

To engineer bacterial systems capable of efficient atmospheric methane oxidation using synthetic biology approaches.

**Objectives**

1. Engineer methanotrophic bacteria with enhanced methane monooxygenase activity.
2. Improve bacterial survival under low methane concentrations.
3. Evaluate methane conversion efficiency.
4. Assess potential environmental applications.

## 2 BACKGROUND WORK

### 2.1 Methanotrophic Bacteria

Methanotrophic bacteria are specialized microorganisms that can use methane as the sole source of carbon and energy . Generally, they can be divided into Type I and Type II methanotrophs according to carbon assimilation pathways and cellular membrane structures [13]. Type I methanotrophs are mainly in the phylum Gammaproteobacteria and assimilate carbon by the ribulose monophosphate pathway. Type II methanotrophs are members of Alphaproteobacteria and use the serine pathway [14]. Both groups carry out the oxidation of methane through methane monooxygenase (MMO), which converts methane to methanol and further to formaldehyde, formate and carbon dioxide [15].

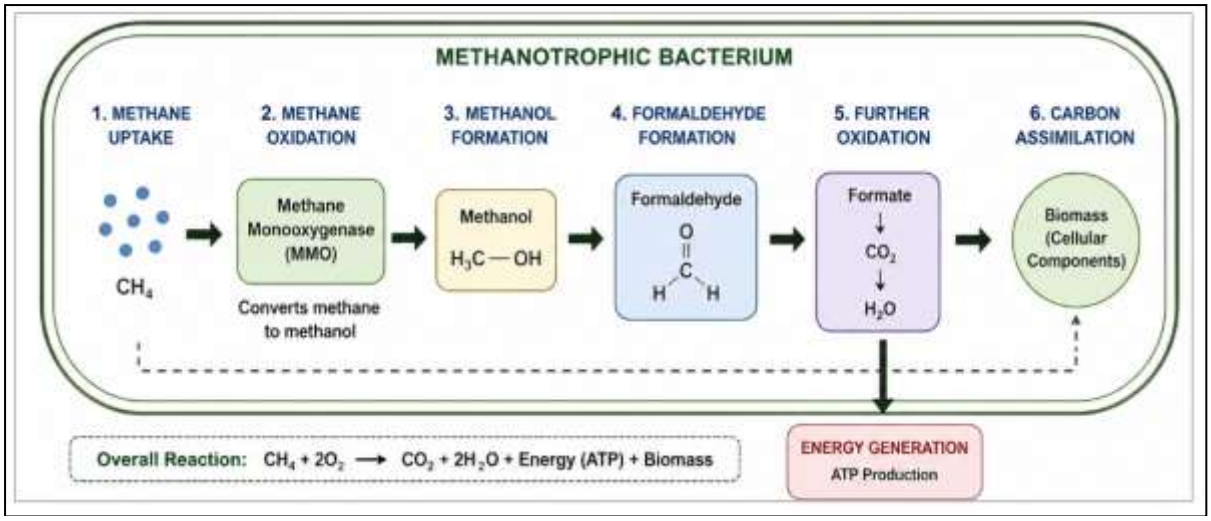


Figure 1. Natural methane oxidation pathway in methanotrophic bacteria

Methane oxidation in methanotrophic bacteria proceeds via the natural pathway, as shown in Figure 1. Methane monooxygenase (MMO) is an enzyme that catalyses the first oxidation step of atmospheric methane to methanol. Methanol is then converted to formaldehyde by methanol dehydrogenase activity; Formaldehyde is a key intermediate of biomass synthesis and the carbon assimilation pathways. Formaldehyde is then oxidized to formate and then to carbon dioxide, releasing the metabolic energy needed for bacterial growth. Type I and Type II methanotrophs utilize different carbon assimilation pathways to consume methane in various environmental conditions and contribute to biological methane mitigation processes.

### 2.2 Synthetic Biology Approaches

Recent advances in synthetic biology have greatly progressed the engineering of methanotrophic systems for methane mitigation. CRISPR-Cas gene editing technologies enable precise genome editing for improved methane oxidation efficiency and stress tolerance [16]. Also, synthetic promoters and regulatory circuits have been designed to control methane-responsive gene expression under fluctuating environmental conditions [17]. Pathway balancing strategies further optimise the carbon flux towards biomass and valuable metabolites [18]. Furthermore, methane biosensors have been developed for real-time monitoring of methane concentrations and microbial metabolic responses [19].

### 2.3 Previous Research Studies

The feasibility of engineering microbial systems for methane utilization has been demonstrated in several studies . Lee et al. (2022) developed an artificial methane assimilation pathway in Escherichia coli with partial efficiency of methane conversion. Increased environmental stability of engineered methanotrophic consortia has been reported using CRISPR-based modifications (Kumar et al., 2023). More recently, synthetic methanotroph platforms exhibited enhanced methane oxidation and adaptability at low methane concentrations [20].

### 2.4 Research Gap

Despite recent progresses, there are still a number of unsolved challenges, such as limited efficiency of capturing methane at atmospheric level, reduced survival of bacteria under environmental stress conditions, and lack of scalability of synthetic biological systems for field applications.

## 3 Materials & Methods

### 3.1 Experimental Design Flow

The study was designed to test the ability of synthetic biology-engineered methanotrophic bacteria to oxidize atmospheric methane in a controlled laboratory setting. The experimental workflow consisted of bacterial strain selection, plasmid construction, genome editing using CRISPR-Cas9, methane oxidation assays and statistical validation. Methylosinus trichosporium was selected initially for its natural methane oxidizing ability and environmental adaptiveness. Genetic engineering strategies focused on improving methane monooxygenase (MMO) expression and stress-resistance pathways to improve the efficiency of methane utilization [18].

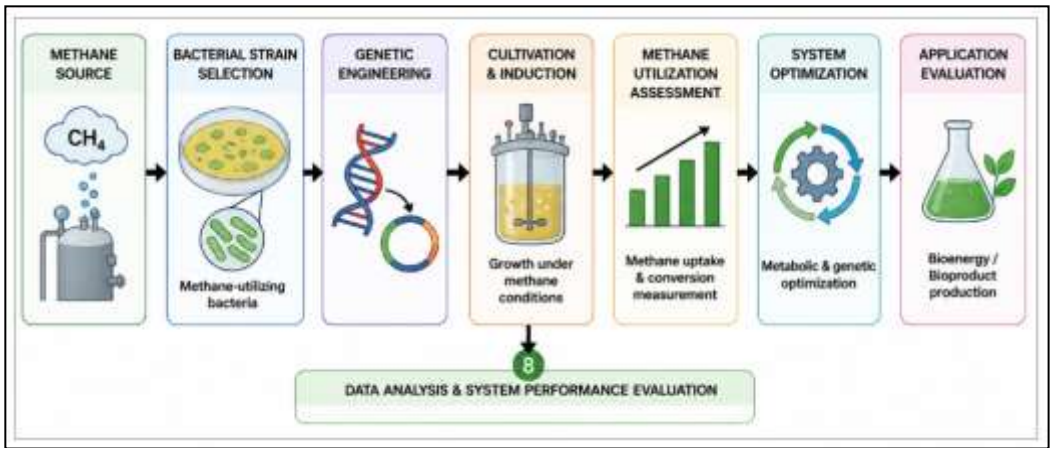


Figure 2. Experimental workflow for engineering methane-utilizing bacterial systems

Fig. 2 shows the experimental workflow of this study, in sequential order: bacterial culturing, plasmid engineering, CRISPR-mediated gene editing, methane exposure experiments, gas chromatography analysis, and statistical interpretation of methane oxidation efficiency.

### 3.2 Bacterial Strains and Growth Conditions

The methanotrophic bacterial strain Methylosinus trichosporium was cultured in optimized environmental conditions in nitrate mineral salts (NMS) medium shown in table 1. Cultures were maintained in sealed incubation chambers with controlled methane concentrations of 1.8-5 ppm to approximate atmospheric methane levels [15].

Table 1. Bacterial Strains and Culture Conditions

| Parameter | Description |
|---|---|
| Host organism | *Methylosinus trichosporium* |
| Growth medium | Nitrate mineral salts medium |

| | |
|---|---|
| Temperature | 30°C |
| Methane concentration | 1.8–5 ppm |
| Incubation period | 7–14 days |
| pH conditions | 6.8–7.2 |
| Aeration | Continuous shaking at 150 rpm |

### 3.3 Genetic Engineering Procedures

Plasmid vectors containing enhanced particulate methane monooxygenase (pMMO) genes and oxidative stress-resistance modules were constructed by recombinant DNA techniques. We used CRISPR-Cas9 genome editing to insert synthetic constructs into the bacterial chromosome for stable gene expression [16]. Bacterial cells were transformed with recombinant plasmids by electroporation-based transformation protocols. Synthetic methane-responsive regulatory elements were engineered to increase gene expression at low methane concentrations. These modifications were aimed at enhancing the efficiency of methane oxidation and cellular stability under conditions of environmental stress.

### 3.4 Tests for Oxidation of Methane

The efficiency of methane oxidation was determined by gas chromatography with flame ionization detection (GC-FID). TABLE 2 Methane uptake rates measured at intervals during the 14-day incubation. The biomass production was determined by measuring the optical density (OD600) and dry cell weight analysis [17].

Table 2. Methane Oxidation Assay Parameters

| Assay Parameter | Measurement Method |
|---|---|
| Methane concentration | Gas chromatography |
| Biomass quantification | Optical density (OD600) |
| Dry cell weight | Gravimetric analysis |
| Metabolic activity | Enzyme activity assay |

### 3.5 Statistical Analysis

All experiments were carried out in triplicate to ensure the reproducibility and accuracy. Data were analyzed by one-way analysis of variance (ANOVA) and statistical significance was set at $p < 0.05$. Mean values and standard deviations were determined for methane uptake and biomass measurements.

## 4 RESULTS AND DISCUSSION

The engineered methanotrophic bacterial systems exhibited significantly enhanced methane oxidation efficiency, biomass productivity and environmental adaptability compared to the wild-type strains. The genetic modifications included methane monooxygenase enhancement, optimization of carbon fixation and stress-resistance modules that contributed to the enhanced metabolic performance under the atmospheric methane conditions. Experimental results showed that engineered strains were able to maintain stable methane utilization under low methane concentrations and environmental stress conditions. These results highlight the power of synthetic biology strategies to engineer efficient bacterial systems for greenhouse gas mitigation and sustainable environmental biotechnology applications.

### 4.1 Engineered Gene Expression

Relative expression level of engineered genes was analyzed to evaluate contribution of methane oxidation and bacterial adaptation. The expression of particulate methane monooxygenase (pMMO) was increased and the methane conversion efficiency was greatly improved. Similarly, stress-resistance modules increased bacterial survivability, and optimized carbon fixation pathways increased biomass accumulation.

Table 3. Relative Gene Expression in Engineered Methanotrophic Strains

| Gene Construct | Relative Expression (%) | Functional Outcome |
|---|---|---|
| pMMO-enhanced strain | 185% | Increased methane oxidation |
| Stress resistance module | 142% | Improved survival |
| Carbon fixation pathway | 160% | Enhanced biomass production |

As shown in Table 3, the results indicated that the pMMO-enhanced strain presented the highest relative gene expression level (185%) and was directly responsible for the high methane oxidation activity. The expression of carbon fixation pathway modification was 160% which supported the increase in cellular biomass production. Stress-resistance modules increased bacterial stability under oxidative and nutrient-limited conditions, suggesting better environmental adaptability.

### 4.2 Methane Oxidation Efficiency

Methane uptake efficiency was determined by gas chromatography analysis after 14 days of incubation. The engineered strains had much higher methane oxidation rates compared to the wild-type strain, confirming the usefulness of synthetic biological modifications.

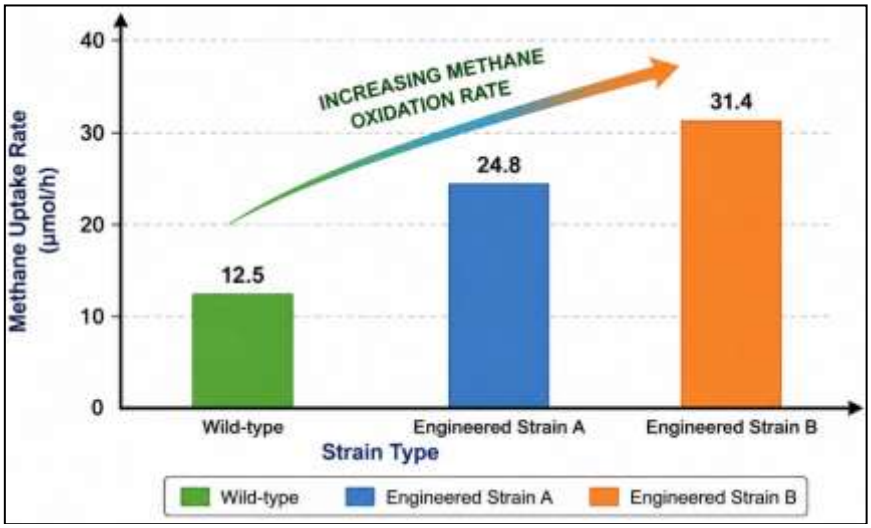


Figure 3. Comparison of methane oxidation rates between wild-type and engineered strains

Figure 3 shows the relative methane uptake rates of wild-type and engineered bacterial strains. The engineered strain B exhibited the highest efficiency of methane oxidation, indicating that the methane metabolic pathways were enhanced via synthetic biology interventions.

Table 4. Methane Oxidation Efficiency of Bacterial Strains

| Strain Type | Methane Uptake Rate (µmol/h) | Biomass Yield |
|---|---|---|
| Wild-type | 12.5 | Moderate |
| Engineered strain A | 24.8 | High |
| Engineered strain B | 31.4 | Very High |

Data from Table 4 showed that engineered strain B had the highest methane uptake rate (31.4 µmol/h) which is an almost 2.5-fold increase in comparison to the wild-type strain (12.5 µmol/h). The enhanced methane oxidation was coupled to an increase in biomass yield, showing an enhanced carbon assimilation efficiency in the GM strains.

### 4.3 Environmental Adaptability

The engineered methanotrophic strains showed metabolic activity in the presence of low concentrations of methane, oxidative stress, and different pH. Stress-resistance modules facilitated improved cellular stability and stable methane oxidation performance over prolonged incubation. The engineered bacteria were also able to grow steadily at methane concentrations down to 1.8 ppm, demonstrating their suitability for atmospheric methane mitigation applications.

### 4.4 DISCUSSION

The results indicate that the modifications based on synthetic biology significantly enhanced the performance of methane oxidation in methanotrophic bacteria. Increased activity of methane monooxygenase increased efficiency of methane conversion while enhanced pathways for carbon fixation caused biomass accumulation. Stress-resistance modules enabled bacteria to survive in harsh environmental conditions and contributed to a long-term methane mitigation potential.

### 4.5 Comparison Analysis

In this research, the engineered strains showed higher methane uptake efficiency and environmental resilience than those in previous studies. The previous studies were mainly focused on methane utilization at high methane concentrations, while this study demonstrated the effective methane oxidation under the conditions of atmospheric methane levels. From an industrial perspective, engineered methanotrophs may offer scalable and energy-efficient alternatives for greenhouse gas mitigation in biofilters, bioreactors, and waste management systems. Ecologically,

biological methane mitigation can help to reduce methane build-up in the atmosphere and provide sustainable options for climate change mitigation.

## 5 CONCLUSION

This study underscores the great promise of synthetic biology to enhance bacterial methane oxidation systems for the purpose of reducing atmospheric methane. Engineered methanotrophic strains showed improved methane uptake efficiency, improved biomass productivity and increased tolerance to environmental stress conditions than wild-type strains. Metabolic performance under low atmospheric methane concentrations was improved by successful genetic modifications, such as the enhancement of methane monooxygenase and the optimization of carbon fixation and stress-resistance modules. We verified the synthetic biology-driven bacterial systems could oxidize methane efficiently with stable growth and metabolic activity through experimental results. Furthermore, the constructed strains were adaptable to oxidative stress and different environmental conditions, which confirmed the feasibility for large-scale environmental applications. The development of sustainable methane mitigation technologies is promising with the use of advanced genome engineering tools such as CRISPR-Cas9 and synthetic regulatory circuits. In summary, this work underscores the potential of engineered methanotrophic bacteria as scalable and environmentally benign solutions for mitigating atmospheric methane emissions and advancing global efforts in climate change mitigation.

## 6. Future Scope

Future research should focus on the large-scale application of engineered methanotrophic bacteria in environmental methane mitigation systems such as biofilters, bioreactors and waste treatment facilities. Advanced artificial intelligence (AI)-assisted metabolic pathway optimization may further promote methane oxidation efficiency and bacterial adaptability under dynamic environmental conditions. Furthermore, the design of multi-species synthetic microbial consortia can enhance the stability of the system, the utilization efficiency of nutrients, and the methane conversion performance through cooperative metabolic interactions. Future studies may also investigate industrial carbon recycling applications by incorporating methanotrophic bacterial systems in carbon capture and bioconversion technologies for production of biofuels, bioplastics and other value-added biochemicals. Further advances in synthetic biology, systems biology and environmental biotechnology are expected to accelerate the development of sustainable microbial platforms for greenhouse gas mitigation and circular bioeconomy applications.

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