



**Politecnico  
di Torino**

**ScuDo**

Scuola di Dottorato ~ Doctoral School

WHAT YOU ARE, TAKES YOU FAR

Doctoral Dissertation  
Doctoral Program in Civil and Environmental Engineering (37<sup>th</sup> Cycle)

# **Unlocking the power of underground (bio)methanation**

**Arianna Vizzarro**

\* \* \* \* \*

## **Supervisors**

Prof. F. Verga

PhD A. Abdel Azim Co-Supervisor

PhD R. Bellini Co-Supervisor

Politecnico di Torino  
2025

This thesis is licensed under a Creative Commons License, Attribution - Noncommercial - NoDerivative Works 4.0 International: see [www.creativecommons.org](http://www.creativecommons.org). The text may be reproduced for non-commercial purposes, provided that credit is given to the original author.

I hereby declare that, the contents and organisation of this dissertation constitute my own original work and does not compromise in any way the rights of third parties, including those relating to the security of personal data.



.....  
Arianna Vizzarro  
Turin, 2025

# Summary

Underground methanation reactor (UMR) technology represents a promising approach for sustainable methane production by leveraging microbial activity in depleted gas reservoirs. This PhD research explores the potential of subsurface environments for biomethanation through a comprehensive investigation, integrating hydrochemical, microbiological, and biotechnological methodologies. The study aimed to characterize suitable reservoirs, optimize microbial enrichment strategies, and assess methanogenic activity under high-pressure conditions.

Firstly, the characterization of two depleted gas reservoirs, R1 and R2, was performed to determine their suitability for UMR application. The analysis of reservoir hydrochemistry and microbial community composition identified R1 as the most promising candidate due to its low salinity, neutral pH, and enriched hydrogenotrophic methanogenic populations. Functional gene analysis and batch experiments confirmed the syntrophic interactions between homoacetogens and methanogens, facilitating efficient methane production. Conversely, R2 exhibited higher salinity and dominant acidogenic activity, leading to less favorable conditions for methanogenesis.

Based on these findings, the best enrichment strategies to stimulate methanogenesis in R1 formation fluids was investigated. The study assessed the effects of various carbon sources, including CO<sub>2</sub>, trypticase peptone, glucose, and acetate, on methane production. Results demonstrated that trypticase peptone significantly enhanced methane yields, together with increased expression of the *mcrA* gene, specific of methanogenic archaea, and a shift in microbial community composition favoring the hydrogenotrophic methanogen *Methanothermobacter*. Functional pathway analysis confirmed the dominance of hydrogenotrophic methanogenesis, with possible syntrophic interactions between fermentative bacteria and methanogens playing a key role in optimizing carbon conversion. These findings highlight the importance of selective carbon source supplementation in enhancing UMR performance.

Pressure dynamics were also investigated to see how they affected methanogenic activity and microbial resilience in high-pressure bioreactors. Experiments were conducted under direct and stepwise pressure regimes to evaluate the adaptability of enriched methanogenic communities. The dominant microorganisms of the enriched consortium identified as *Methanothermobacter wolfeii* through whole-genome sequencing (WGS), demonstrated significant resilience to direct pressure increases, suggesting that bioaugmentation with pressure tolerant methanogens could be a strategy to enhance methane production in UMRs. Additionally, a comparative analysis of sequencing methodologies (16S rRNA V3-V4, full-length 16S rRNA, and WGS) provided valuable insights into the accuracy and resolution of microbial community profiling. The study underscores the importance of methodological choice in sequencing for ecological and biotechnological research.

Overall, this research demonstrates the critical importance of strategic reservoir selection, targeted microbial enrichment, and adaptive bioaugmentation for optimizing UMR technology. The findings underscore the potential of depleted gas reservoirs as bioreactors for methane production and emphasize the necessity of integrated biological, technical, and computational approaches to enhance microbial performance.