

Pressure and Trace Metal Optimization of Hydrogenotrophic Biomethanation Using Response Surface Methodology

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Biological methanation via hydrogenotrophic archaea represents a promising Power-to-Gas (PtG) pathway for converting renewable hydrogen (H₂) and carbon dioxide (CO₂) into methane (CH₄) under mild operational conditions (Rusmanis et al., 2019). This process enables efficient energy storage, carbon recycling, and direct integration into existing natural gas infrastructure. Despite its potential, the scalability and efficiency of biological methanation are constrained by two major factors: (i) the inherently low aqueous solubility of hydrogen, which limits gas–liquid mass transfer, and (ii) insufficient understanding of the combined effects of operational pressure and trace metal availability on methanogenic activity. Trace metals such as iron (Fe), nickel (Ni), and cobalt (Co) are essential cofactors in key enzymatic systems involved in hydrogenotrophic methanogenesis, yet their optimal concentrations under pressurized conditions remain poorly defined (Ebrahimian et al. 2023).

The present study systematically investigates the synergistic effects of applied pressure and trace metal supplementation on biological methanation performance using Response Surface Methodology (RSM). To our knowledge, this is the first work that simultaneously evaluates these physicochemical and biochemical parameters within a unified experimental and statistical framework. The primary objectives were to optimize methane production efficiency and minimize conversion time by identifying optimal combinations of pressure, Fe(II), Ni(II), and Co(II) concentrations.

Batch biomethanation experiments were conducted in custom-made, gas-tight reactors operated under thermophilic conditions (55°C) with continuous magnetic stirring to ensure homogeneous mixing. The reactors were inoculated with a hydrogenotrophic methanogenic culture enriched from a pilot-scale trickle bed reactor, characterized by enhanced gas–liquid mass transfer and biofilm-based microbial immobilization. System overpressure was maintained using inert nitrogen gas, while Fe(II), Ni(II), and Co(II) were supplied as chloride salts to support enzymatic activity.

A face-centered composite design (FCC) was employed to explore the experimental domain, comprising 52 runs with four independent variables: applied pressure (0.5–2 bar), Fe(II) concentration (1–50 mg L⁻¹), Ni(II) concentration (0.01–0.5 mg L⁻¹), and Co(II) concentration (0.01–0.1 mg L⁻¹). Two key response variables were evaluated: (i) H₂/CO₂ conversion efficiency (%) and (ii) conversion time (h). Gas composition was monitored daily using gas chromatography, while process completion was tracked via pressure decay measurements. Statistical analysis was performed using Design-Expert® software, including analysis of variance (ANOVA), model diagnostics, and optimization routines.

Quadratic polynomial models were developed for both response variables and demonstrated strong predictive performance. The model for conversion time exhibited a coefficient of determination (R²) of 0.9458, while the conversion efficiency model achieved an R² of 0.9204,

both with high F-values and p-values < 0.0001, confirming statistical significance. Diagnostic analyses confirmed normality, independence, and homoscedasticity of residuals, validating the robustness of the models.

The results identified applied pressure as the most influential parameter, significantly enhancing hydrogen mass transfer and reducing conversion time by up to 38%. Among the trace metals, Fe(II) and Ni(II) exerted a pronounced positive effect on both methane production rate and overall conversion efficiency, reflecting their central role in hydrogenase and methyl-coenzyme M reductase systems. Co(II) contributed to process performance, but its effect was less consistent and primarily manifested through interaction terms rather than as an independent factor. Notably, significant interaction effects between pressure and trace metal concentrations were observed, underscoring the importance of multivariate optimization approaches over conventional one-factor-at-a-time methodologies.

Numerical optimization using RSM identified optimal operational conditions at 1.25 bar pressure, 30 mg L⁻¹ Fe(II), 0.1 mg L⁻¹ Ni(II), and 0.045 mg L⁻¹ Co(II). Under these conditions, the model predicted a methane conversion efficiency of 97.4% and a conversion time of 16.9 hours. Validation experiments conducted within the design space confirmed the accuracy of the model, yielding experimental values of 95.1% conversion efficiency and 17.3 hours conversion time, corresponding to prediction errors below 5%.

In conclusion, this study demonstrates that the combined optimization of pressure and trace metal supplementation can substantially enhance biological methanation performance by overcoming both mass transfer and metabolic limitations. The application of RSM provided a reproducible and statistically sound framework for identifying optimal operating conditions, offering valuable insights for the design and scale-up of high-efficiency biomethanation systems. These findings support the integration of biological methanation into renewable energy infrastructures and contribute to advancing sustainable carbon utilization technologies.

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