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

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Introduction

Biological methanation is a microbially-mediated process where archaea convert H_2 and CO_2 into CH_4 and water under mild conditions (35-55°C, 1-5 bar), presenting an energy-efficient alternative to thermocatalytic methods. The process faces a critical limitation in H_2 mass transfer due to its low aqueous solubility (1.44 mg/L at 50°C), which can be improved through elevated pressure to enhance solubility and diffusion rates. Equally crucial are trace metals (Fe(II), Ni(II), Co(II)) that serve as enzymatic cofactors for methanogenic pathways. While Response Surface Methodology (RSM) has been applied to study trace metals or pressure effects independently in anaerobic digestion, the potential synergistic interaction between these parameters remains unexplored in biological methanation systems. This knowledge gap represents a significant opportunity, as understanding these combined effects could lead to potentially unlocking new efficiencies in reactor design and operation.

This study uses Response Surface Methodology to optimize biomethanation by analyzing how trace metals (Fe, Ni, Co) and pressure affect gas conversion rates and speed. The approach evaluates both individual and combined effects of these factors to find the best operating conditions. This method helps maximize efficiency while reducing processing time compared to conventional single-factor testing.

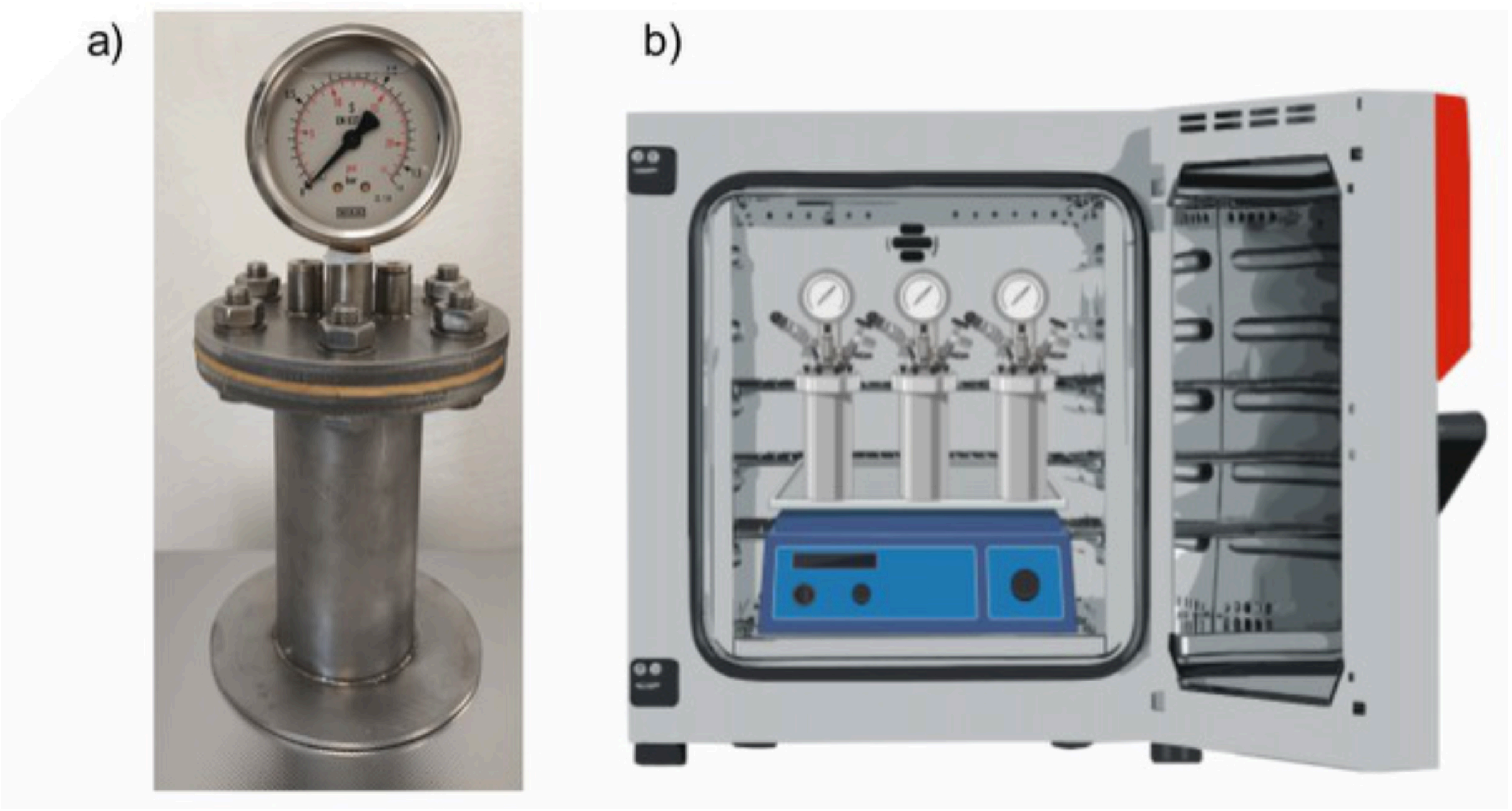

Figure 1. (a) The batch reactor utilized in this study. (b) Schematic representation of the batch reactors placed on a magnetic stirrer and inside a laboratory air oven.

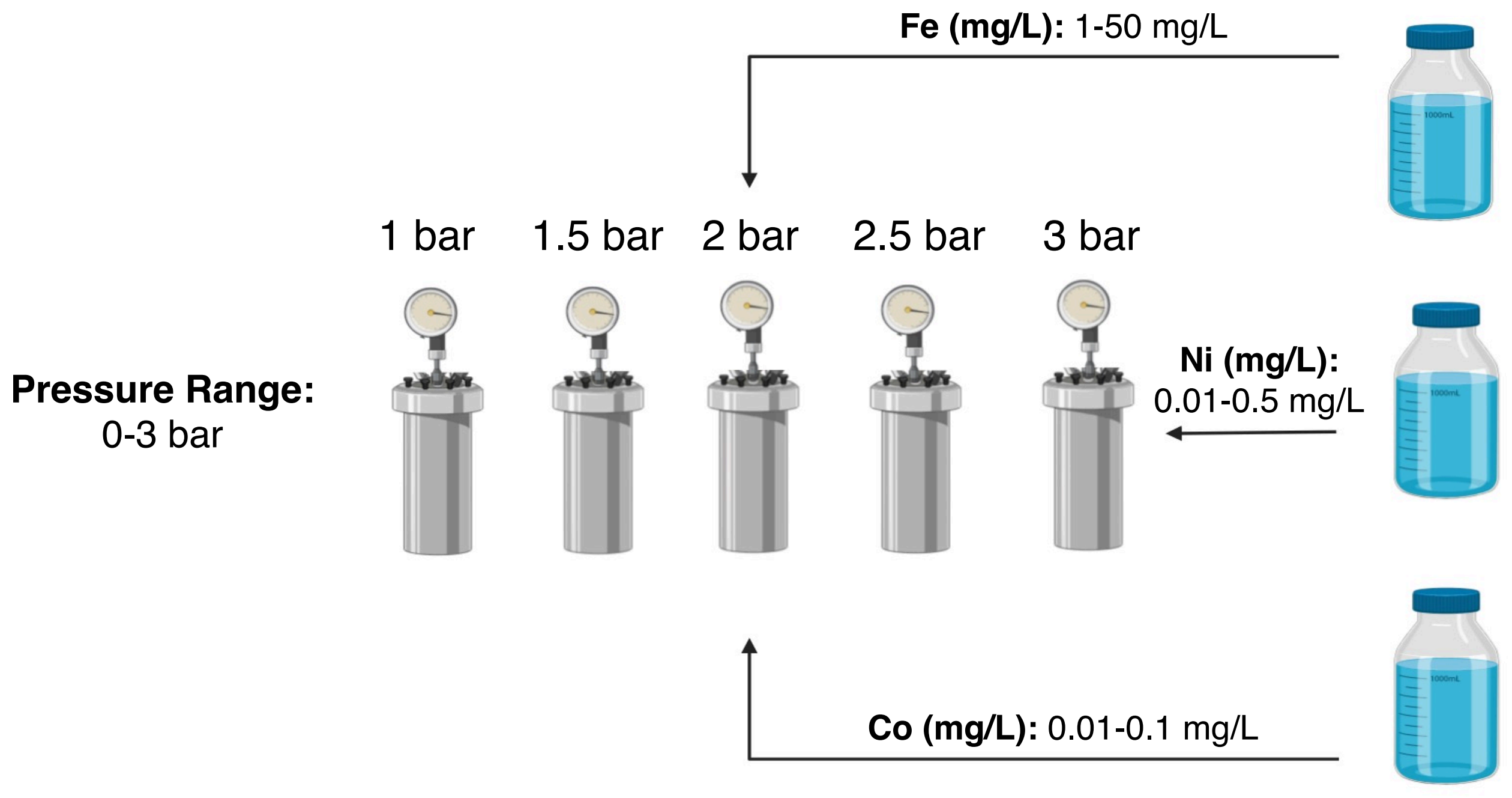

Figure 2. Experimental conditions of the study, ie. pressure and nutrient concentration values.

References

Rusmanis, D.; O'Shea, R.; Wall, D.M.; Murphy, J.D. Biological Hydrogen Methanation Systems – an Overview of Design and Efficiency. Bioengineered 2019, 10, 604–634, doi:10.1080/21655979.2019.1684607.

Bernhardt, G.; Jaenicke, R.; Lüdemann, H.D.; König, H.; Stetter, K.O. High Pressure Enhances the Growth Rate of the Thermophilic Archaebacterium Methanococcus Thermolithotrophicus without Extending Its Temperature Range. Appl Environ Microbiol 1988, 54, 1258–1261, doi:10.1128/aem.54.5.1258-1261.1988.

Materials & Methods

Five pressurized batch reactors (55°C) with magnetic stirring tested biomethanation using a face-centered composite design (52 runs) varying Fe/Ni/Co concentrations (1-50, 0.01-0.5, 0.01-0.1 mg/L) and pressure (0.5-2 bar). Gas composition was analyzed by GC, with conversion efficiency and time as key responses. Statistical analysis (ANOVA, $p<0.05$) evaluated parameter effects on H_2/CO_2 conversion. The model's accuracy was verified through prediction error calculations between experimental and predicted values.

ANOVA analysis showed pressure (X_A), iron (X_B), and nickel (X_C) significantly affected conversion time (Y1) ($p<0.001$), while cobalt (X_D) had minimal impact. For conversion rate (Y_2), pressure (X_A), iron (X_B), and cobalt (X_D) were significant, unlike nickel (X_C). The Y_1 quadratic model showed strong predictability ($R^2=0.9458$, $F=75.23$, $p<0.0001$) with high precision (31.78) and low variation (CV=8.39%) (Equation 1). Similarly, the Y_2 model demonstrated excellent fit ($R^2=0.9204$, $F=59.97$, $p<0.0001$) with precise predictions (27.78) and minimal variability (CV=2.10%) (Equation 2). Residual analysis confirmed all regression assumptions were valid.

$$\text{Conversion Time (h)}^{-1.49} = -0.007228 + 0.016332\ \text{Pressure} + 0.000291\ \text{Fe} + 0.018808\ \text{Ni} + 0.084076\ \text{Co} - 0.000133\ \text{Pressure} * \text{Fe} - 0.002103\ \text{Pressure} * \text{Ni} - 0.030902\ \text{Pressure} * \text{Co} - 0.000506\ \text{Fe} * \text{Co} + 0.083651\ \text{Ni} * \text{Co} - 0.002686\ \text{Pressure}^2 - 0.032734\ \text{Ni}^2 - 0.492681\ \text{Co}^2 \quad \textbf{Eq.}\ (1)$$

$$\text{Conversion Rate (\%)} = 60.49469 + 28.21056\ \text{Pressure} + 0.58222\ \text{Fe} + 0.648526\ \text{Ni} + 136.19035\ \text{Co} - 0.173639\ \text{Pressure} * \text{Fe} - 3.34694\ \text{Pressure} * \text{Ni} - 59.62693\ \text{Pressure} * \text{Co} - 0.789116\ \text{Fe} * \text{Co} - 5.06282\ \text{Pressure}^2 - 0.03195\ \text{Fe}^2 \quad \textbf{Eq.}\ (2)$$

Results & Discussion

The results identified pressure and Fe(II)/Ni(II) concentrations as the most influential factors affecting biomethanation performance. Quadratic modeling exhibited strong predictive accuracy, with perturbation analysis highlighting pressure as the dominant factor, primarily due to its role in enhancing hydrogen mass transfer reducing conversion time by up to 38%. Trace metal supplementation, particularly Fe(II) and Ni(II), was found to significantly enhance enzymatic activity, supporting microbial efficiency. Response surface methodology identified optimal conditions at 1.25 bar pressure, with 30 mg/L Fe(II) and 0.1 mg/L Ni(II), achieving 97.4% methane purity within 16.9 hours. These conditions were validated experimentally, with a prediction error of less than 5%.

Conclusions

- Synergistic optimization of pressure (0.5–2 bar) and trace metals (Fe/Ni/Co) enables >95% substrate conversion efficiency in biomethanation.
- RSM models demonstrate high reliability ($R^2>0.92$) for predicting both conversion time (Y_1) and rate (Y_2), with pressure (X_A) and iron (X_B) as dominant control factors.

Acknowledgement:
The project is co-funded by LIFE, the EU's financial instrument supporting environmental nature conservation and climate action projects throughout the EU.