

Supplementary Material

Harnessing the influence of pressure and nutrients on biological CO₂ methanation using Response Surface Methodology and Artificial Neural Network—Genetic Algorithm approaches

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S1. Growth Medium Composition

The following stock solutions were used for the preparation of the BA:

- Solution A contained NH_4Cl — $100 \text{ g}\cdot\text{l}^{-1}$, NaCl — $10 \text{ g}\cdot\text{l}^{-1}$, $\text{MgCl}_2\cdot 6\text{H}_2\text{O}$ — $10 \text{ g}\cdot\text{l}^{-1}$ and $\text{CaCl}_2\cdot 2\text{H}_2\text{O}$ — $5 \text{ g}\cdot\text{l}^{-1}$;
- Solution B contained $\text{K}_2\text{HPO}_4\cdot 3\text{H}_2\text{O}$ — $200 \text{ g}\cdot\text{l}^{-1}$;
- Solution C contained NaHCO_3 — $52 \text{ g}\cdot\text{l}^{-1}$;
- Trace Metal Solution (TMS)(D) contained $\text{FeCl}_2\cdot 4\text{H}_2\text{O}$ — $2 \text{ g}\cdot\text{l}^{-1}$, H_3BO_3 — $0.05 \text{ g}\cdot\text{l}^{-1}$, ZnCl_2 — $0.05 \text{ g}\cdot\text{l}^{-1}$, CuCl_2 — $0.03 \text{ g}\cdot\text{l}^{-1}$, $\text{MnCl}_2\cdot 4\text{H}_2\text{O}$ — $0.05 \text{ g}\cdot\text{l}^{-1}$, $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot 4\text{H}_2\text{O}$ — $0.05 \text{ g}\cdot\text{l}^{-1}$, AlCl_3 — $0.05 \text{ g}\cdot\text{l}^{-1}$, $\text{CoCl}_2\cdot 6\text{H}_2\text{O}$ — $0.05 \text{ g}\cdot\text{l}^{-1}$, H_2SeO_3 — $0.05 \text{ g}\cdot\text{l}^{-1}$;
- Vitamin Solution (VS)(E) contained vitamins B7— $20 \text{ mg}\cdot\text{l}^{-1}$, B6— $100 \text{ mg}\cdot\text{l}^{-1}$, B2— $50 \text{ mg}\cdot\text{l}^{-1}$, B1— $50 \text{ mg}\cdot\text{l}^{-1}$, B12— $0.1 \text{ mg}\cdot\text{l}^{-1}$, folic acid— $20 \text{ mg}\cdot\text{l}^{-1}$, nicotinic acid— $50 \text{ mg}\cdot\text{l}^{-1}$, P-aminobenzoic acid— $50 \text{ mg}\cdot\text{l}^{-1}$, thiotic acid— $50 \text{ mg}\cdot\text{l}^{-1}$ and DL-pantothenic acid— $5 \text{ mg}\cdot\text{l}^{-1}$; and
- Solution F contained Na_2S — $25 \text{ g}\cdot\text{l}^{-1}$. Solution F was prepared anaerobically in an anaerobically sealed serum vial by adding Na_2S to distilled H_2O after flushing for 20 min with N_2 to remove solubilized O_2 .

The volumes of each stock solution per liter of medium were: A—10 ml, B—2 ml, C—50 ml, D—1 ml, E—1 ml, and F—10 ml. The stock solutions were added in 936 ml of distilled H₂O.

Tables

Table S1. Range of independent variables chosen for the FCC design.

Factor	Units	Coded and actual values		
		-1 (Low)	0 (Center point)	+1 (High)
Pressure, X_A	bar	0.5	1.25	2.0
Fe (II), X_B	mg/L	1.0	25.5	50.0
Ni(II), X_C	mg/L	0.01	0.255	0.50
Co(II), X_D	mg/L	0.01	0.055	0.10

Table S2. Performance metrics (MSE, RMSE, and R^2) for artificial neural network models with varying numbers of hidden layer neurons.

Hidden layer size	ANN model	MSE	RMSE	R^2
1	4-1-2	0.0029	0.0539	0.9833
2	4-2-2	0.0063	0.0794	0.9669
3	4-3-2	0.0014	0.0374	0.9922
4	4-4-2	0.0018	0.0424	0.9910
5	4-5-2	0.0011	0.0332	0.9938
6	4-6-2	0.0008	0.0283	0.9950
7	4-7-2	0.0006	0.0245	0.9968
8	4-8-2	0.0017	0.0412	0.9916
9	4-9-2	0.0023	0.0480	0.9866
10	4-10-2	0.0017	0.0412	0.9924
11	4-11-2	0.0037	0.0608	0.9803
12	4-12-2	0.0009	0.0301	0.9920
13	4-13-2	0.0018	0.0424	0.9898
14	4-14-2	0.0051	0.0714	0.9700
15	4-15-2	0.0040	0.0632	0.9825
16	4-16-2	0.0026	0.0510	0.9853
17	4-17-2	0.0017	0.0412	0.9908
18	4-18-2	0.0151	0.1229	0.9247
19	4-19-2	0.0013	0.0361	0.9930
20	4-20-2	0.0025	0.0500	0.9876

Table S3. Comparison of model fitting characteristics between FCC-RSM and ANN-GA for the two responses.

Method	Model	Statistical Parameters						Optimization method
		R ²		MSE		RMSE		
		Time (h)	Conversion (%)	Time (h)	Conversion (%)	Time (h)	Conversion (%)	
RSM	Second-order quadratic equation	0.9586	0.9360	5.9418	3.0804	2.4376	1.7551	Numerical optimization
ANN- GA	4–7–2 Artificial neural network		0.9968		0.0006		0.0245	Genetic Algorithm

Figures

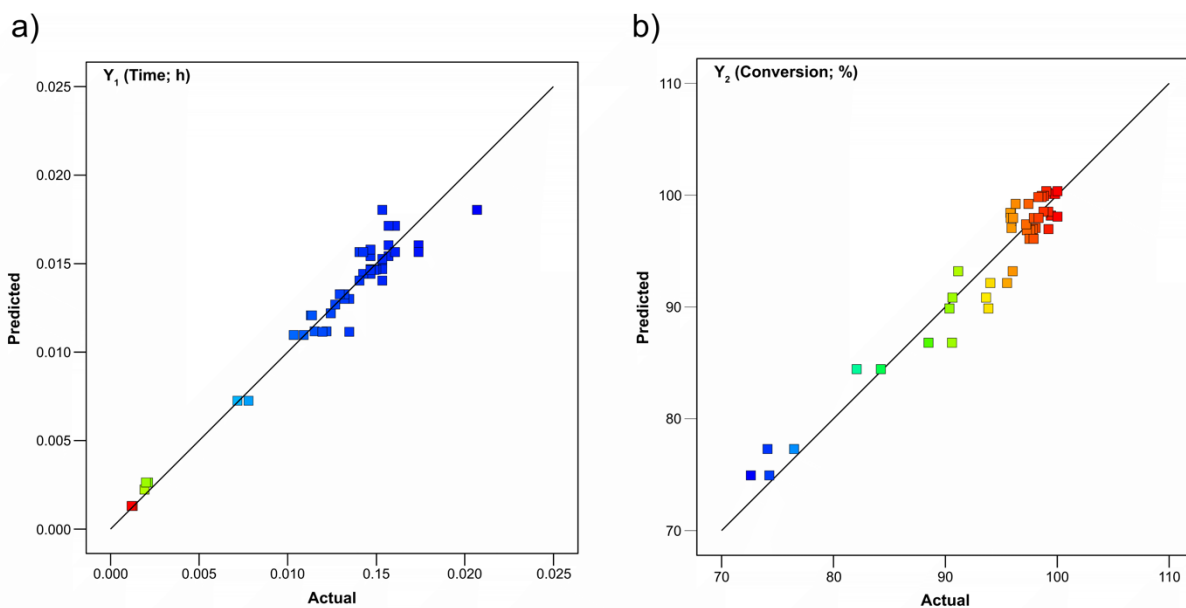


Figure S1. Correlation between predicted and experimental values for (a) conversion time (h) Y_1 ; (b) conversion rate (%) Y_2 .

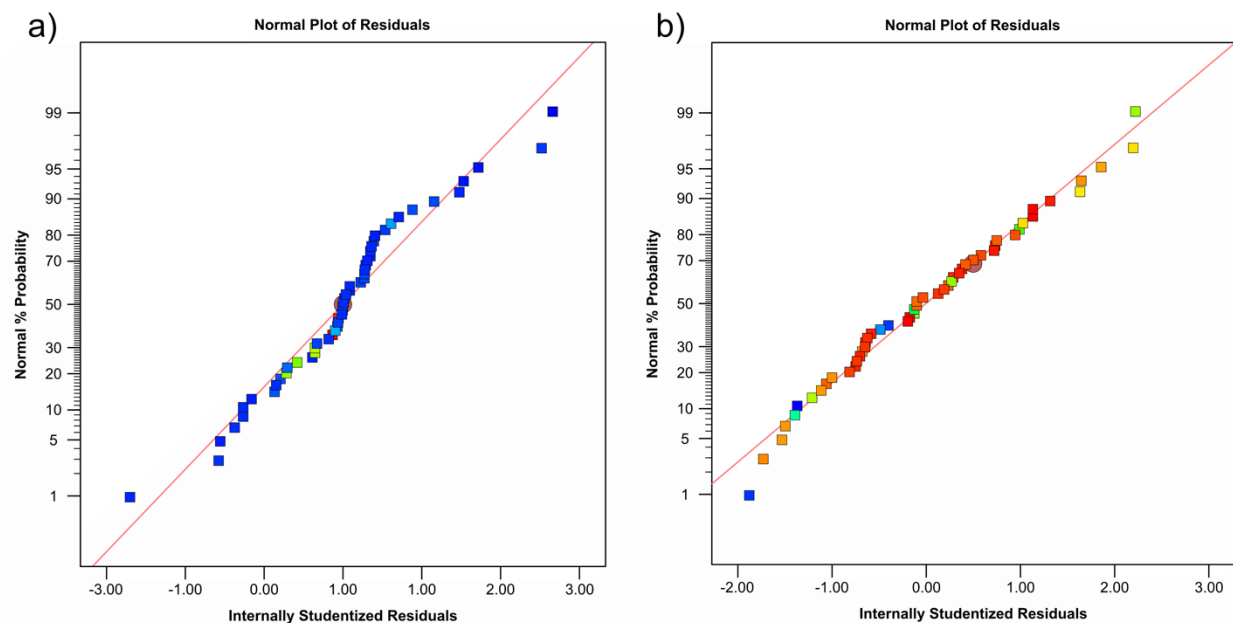


Figure S2. Normal probability plots of internally studentized residuals for (a) conversion time (h) (Y_1); (b) conversion rate (%) (Y_2).

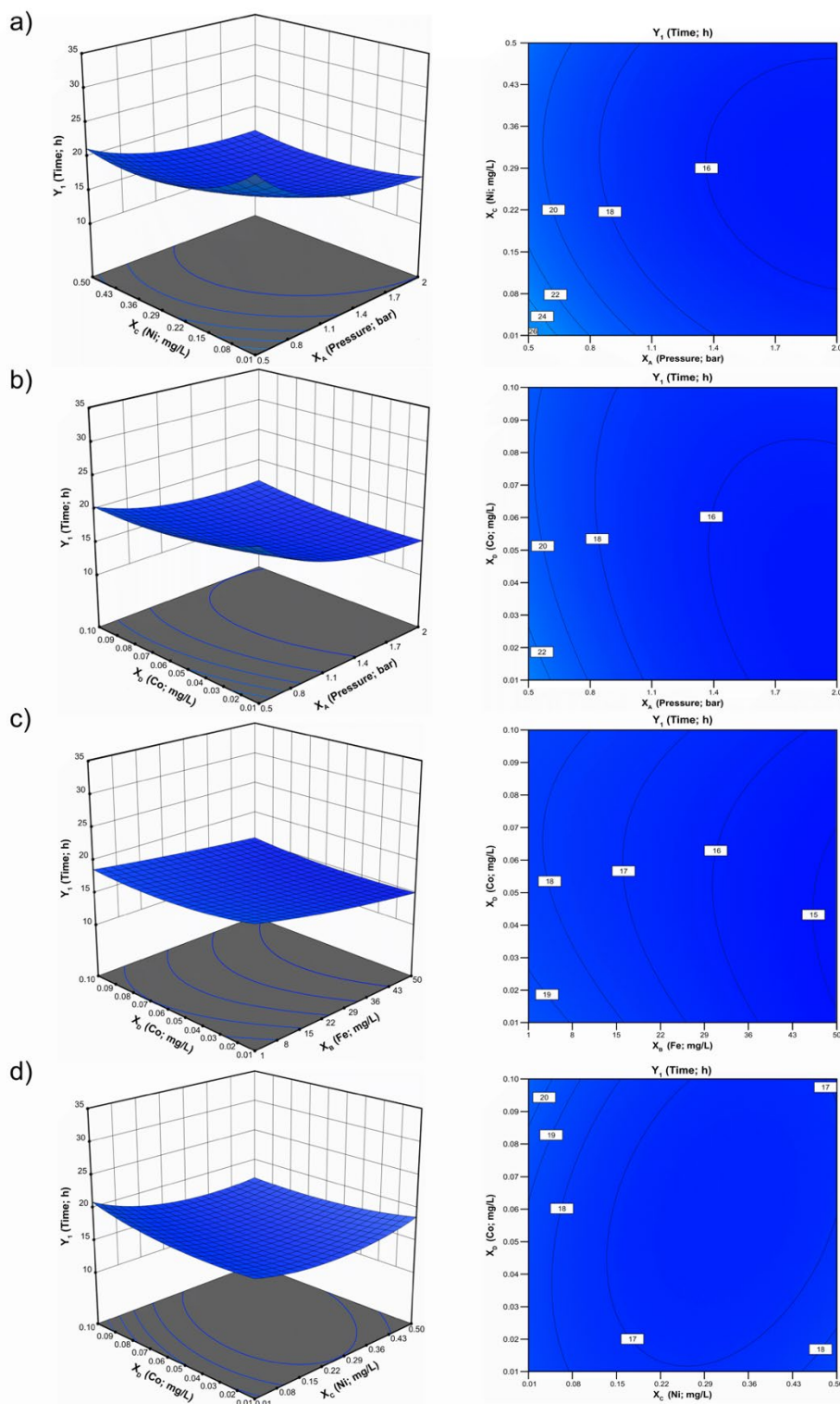


Figure S3. Response surface and contour plots for conversion time (h), as a function of: (a) pressure (bar) (X_A) and Ni(II) concentration (mg/L) (X_C), (b) pressure (bar) (X_A) and Co(II) concentration (mg/L) (X_D), (c) Fe(II) concentration (mg/L) (X_B) and Co(II) concentration (mg/L) (X_D), and (d) Ni(II) concentration (mg/L) (X_C) and Co(II) concentration (mg/L) (X_D).

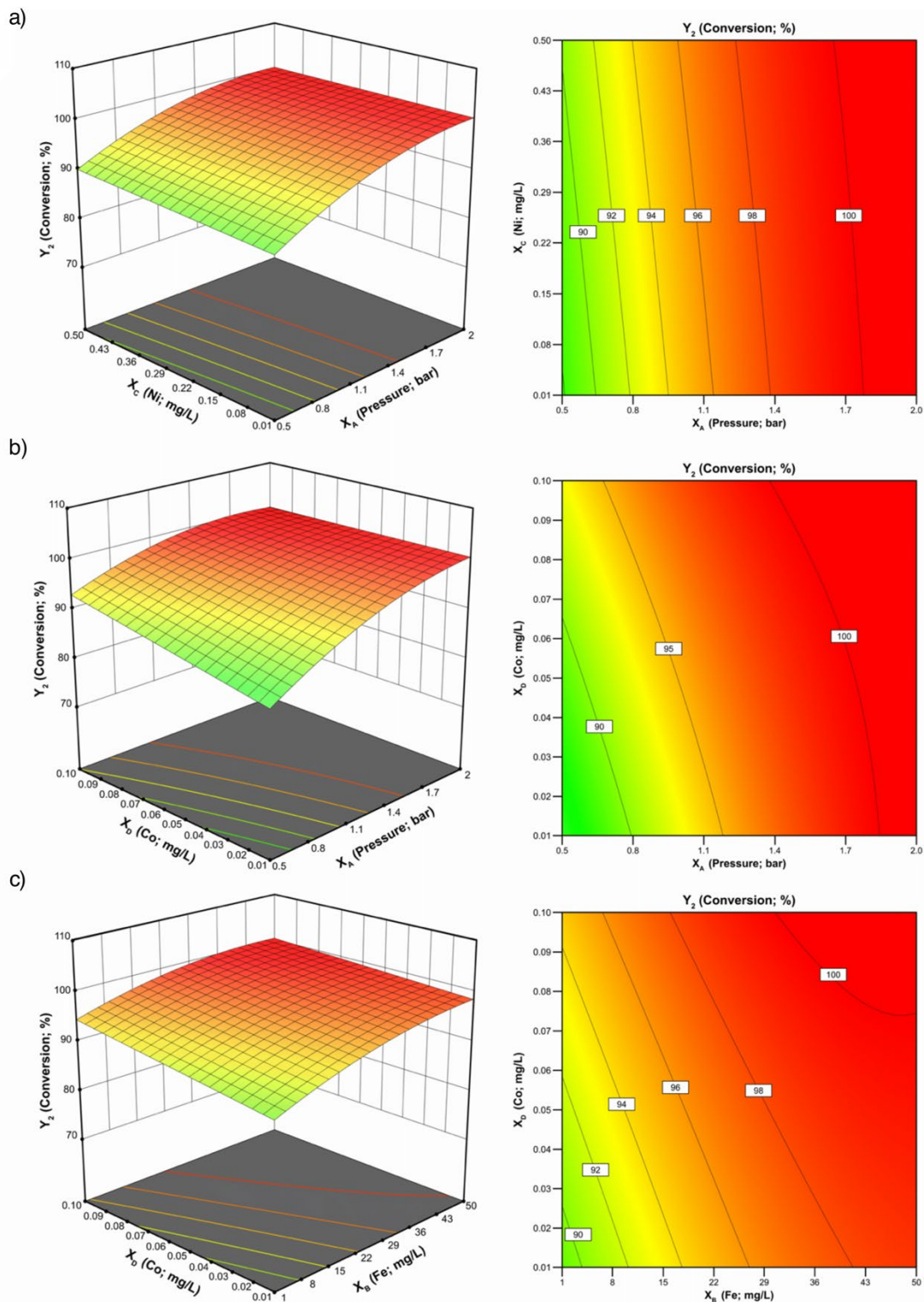


Figure S4. Response surface and contour plots for conversion rate (%), as a function of: (a) pressure (bar) (X_A) and Co(II) concentration (mg/L) (X_D), (b) pressure (bar) (X_A) and Co(II) concentration (mg/L) (X_D), and (c) Fe(II) concentration (mg/L) (X_B) and Co(II) concentration (mg/L) (X_D).

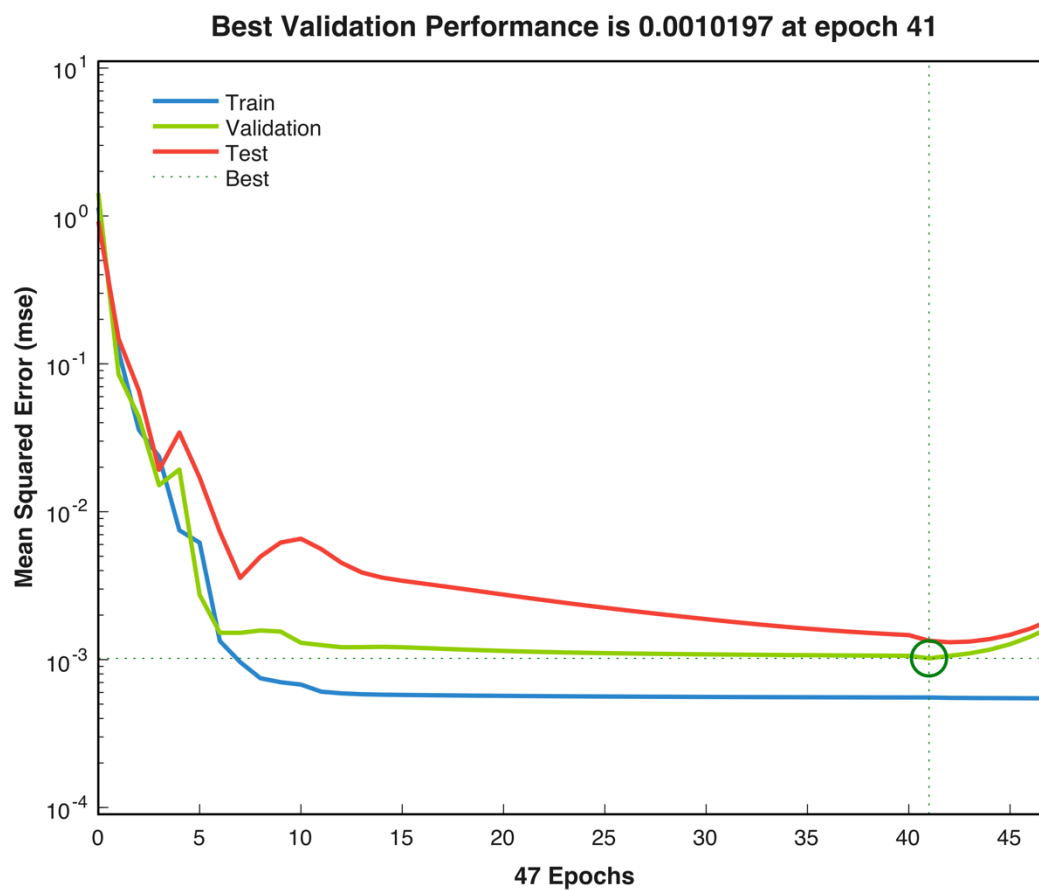


Figure S5. Validation performance over epochs of the trained ANN for the two output responses.