

Supporting Information

CO₂-Driven pH Control in the Enzymatic Hydrolysis of Urea: A Coupled Modelling–Experiment Approach

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This Supplementary Information file contains numerical values of the data points presented in the main publication. This dataset and the Python code are available through TORE together with a descriptive README file: <https://doi.org/10.15480/882.17011>.

The following sections contain information on the calibration curve for the ammonia test kit (S1), urea HPLC analytics (S2), pH analysis (S3), ammonia testkit results (S4), urea HPLC results (S5), the respective HPLC chromatograms (S6), parameter fits for the kinetic models (S7), S8: Results of the Bayesian optimization for the two experimentally validated points (S8) and the experimental results for the two experimentally validated points (S9)

S1: Calibration curve for ammonia test kits

Table S1 – Ammonia calibration curve using ammonia test kits. Results are presented as mean \pm the standard deviation.

Concentration (mg/L)	Absorbance at 660 nm
5	0.0725 \pm 0.0006
15	0.1237 \pm 0.0001
25	0.1872 \pm 0.0005
35	0.2430 \pm 0.0046
45	0.3243 \pm 0.0053
50	0.33927 \pm 0.0014

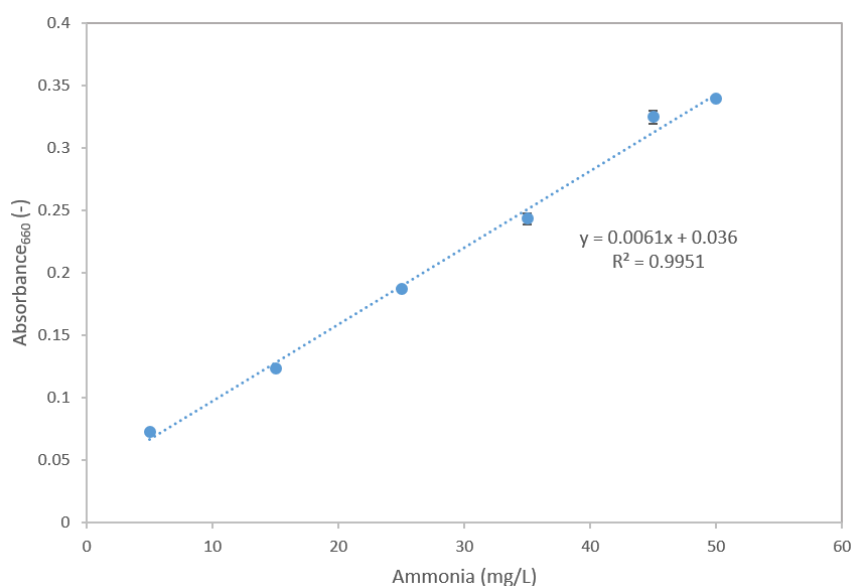


Figure S1 - Calibration curve of ammonia using ammonia test kits. Shown as mean \pm standard deviation.

S2: Calibration Curve for urea in HPLC

Table S2 - Urea calibration curve in the HPLC at 0.5 mL/min flowrate with double distilled water as eluent. Results are presented as mean \pm the standard deviation. Measurements are independent triplicates.

Concentration (mM)	Peak Area (-)
3	782.0 \pm 58.4
6	1522.8 \pm 73.4
9	2241.7 \pm 120.5
12	2818.2 \pm 21.4
15	3402.5 \pm 116.4
18	4073.9 \pm 65.4
21	4493.4 \pm 136.4
24	5313.5 \pm 115.3
27	5677.3 \pm 170.6
30	6303.3 \pm 94.9

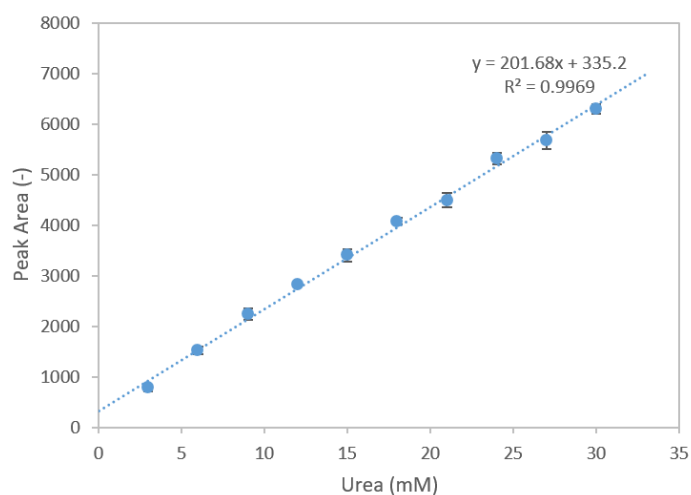


Figure S2 - Calibration curve of urea in the HPLC. Shown as mean \pm standard deviation.

Table S3 - Values for the linear regression of the HPLC calibration curve.

Standard error (-)	108.28
Intercept	335.20
Slope	201.68
R ²	0.997
LOD (mM)	1.77
LOQ (mM)	5.37

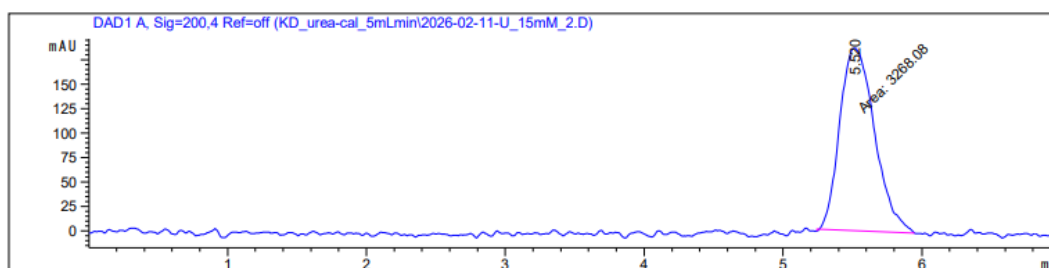


Figure S3 - Example of chromatogram at 200 nm for 15 mM urea.

S3: pH results

Table S4 - pH of the reaction solution after 1 h of reaction, measured directly after opening the reactors. Results are presented as mean \pm the standard deviation. Measurements are independent triplicates.

Pressure (bar)	pH (-)
0	9.05 \pm 0.03
0.5	7.95 \pm 0.08
1	7.80 \pm 0.10
2.1	7.10 \pm 0.22
3.5	6.92 \pm 0.09

S4: Ammonia test kit results

Table S5 - Final urea concentration of the reaction solution after 1 h of reaction, calculated from measuring with ammonia test kits. Results are presented as mean \pm the standard deviation. Measurements are independent triplicates.

Pressure (bar)	Urea (mM)
0	26.1 \pm 0.7
0.5	22.3 \pm 1.6
1	7.8 \pm 1.4
2.1	7.1 \pm 4.3
3.5	6.9 \pm 2.0

S5: HPLC results

Table S6 - Final urea concentration of the reaction solution after 1 h of reaction, measured using HPLC. Results are presented as mean \pm the standard deviation. Measurements are independent triplicates.

Pressure (bar)	Urea (mM)
0	26.7 \pm 1.9
0.5	18.7 \pm 3.3
1	10.2 \pm 0.6
2.1	11.7 \pm 1.3
3.5	8.9 \pm 0.6
	9.9 \pm 1.9

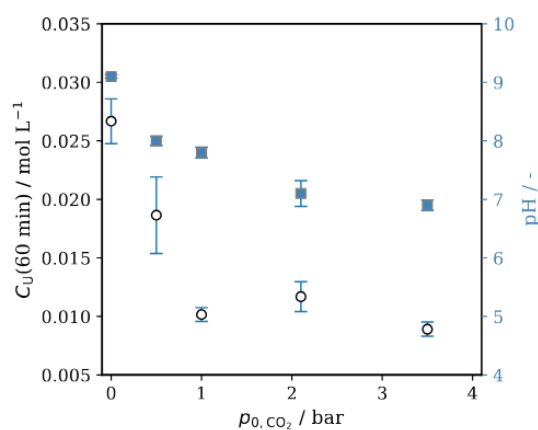
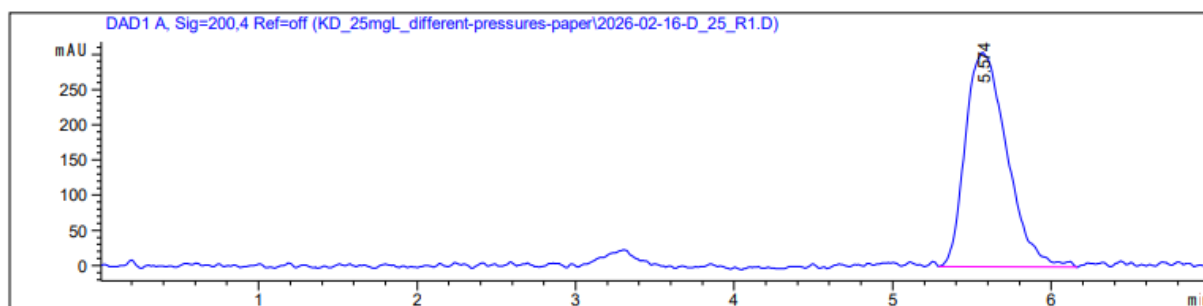


Figure S4 - Dependency of final urea concentrations after a 60 minute reaction on the CO_2 overpressure in the reactor using HPLC method. Results are shown as mean \pm standard deviation of experimental triplicates ($n=3$).

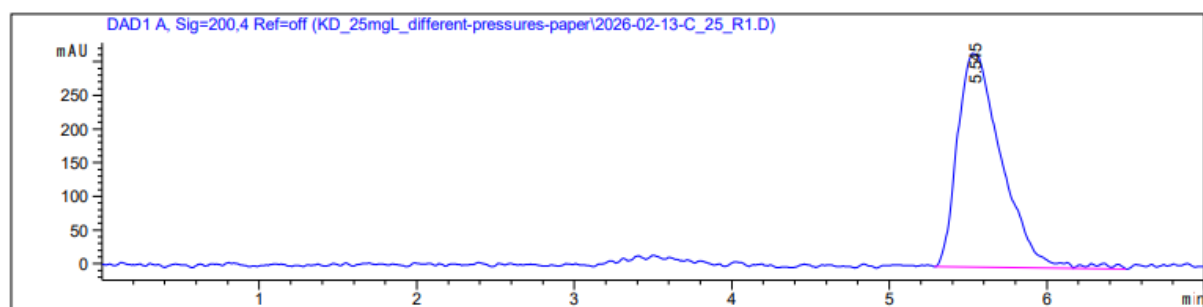
S6: HPLC chromatograms

a. 0 bar pressure in the reactor



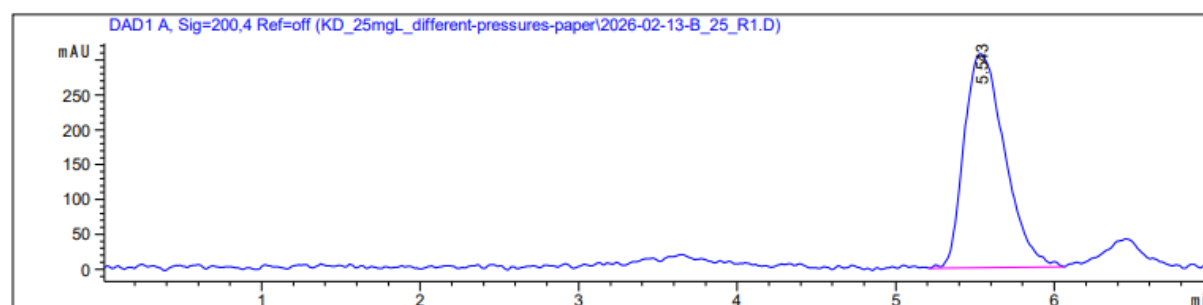
RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [mM]	Grp	Name
5.574	BB	5554.19189	4.64566e-3	25.80289		Urea

Figure S5 - HPLC chromatogram for no pressure – 1st replicate.



RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [mM]	Grp	Name
5.545	BB	6183.48291	4.66824e-3	28.86598		Urea

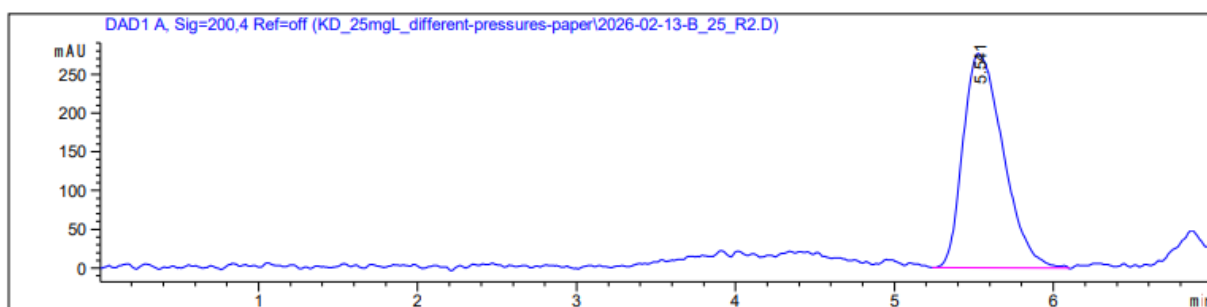
Figure S6 - HPLC chromatogram for no pressure – 2nd replicate.



RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [mM]	Grp	Name
5.543	BB	5467.38818	4.64214e-3	25.38036		Urea

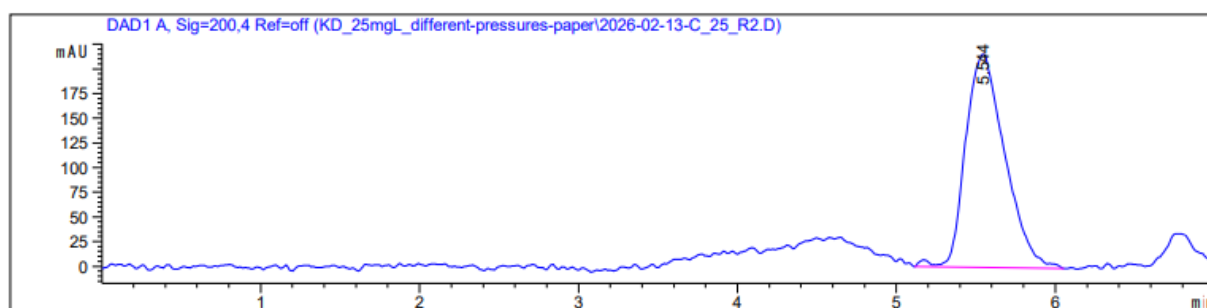
Figure S7 - HPLC chromatogram for no pressure – 3rd replicate.

b. 0.5 bar pressure in the reactor



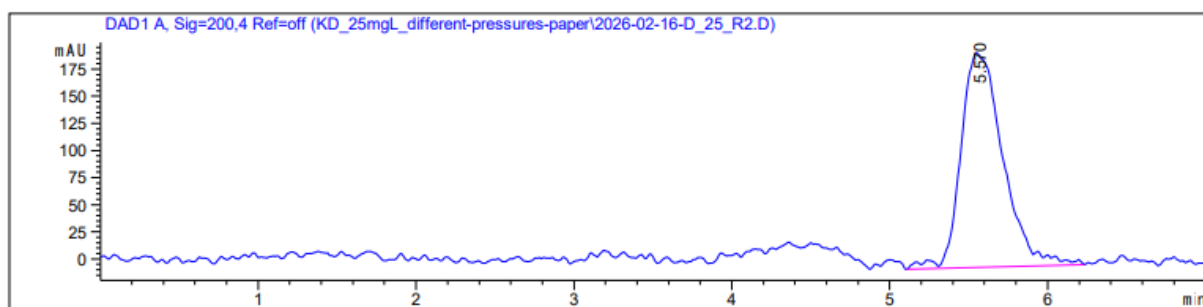
RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [mM]	Grp	Name
5.541	BB	4858.70996	4.61390e-3	22.41760		Urea

Figure S8 - HPLC chromatogram for 0.5 bar pressure – 1st replicate.



RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [mM]	Grp	Name
5.544	BB	3726.28296	4.53682e-3	16.90547		Urea

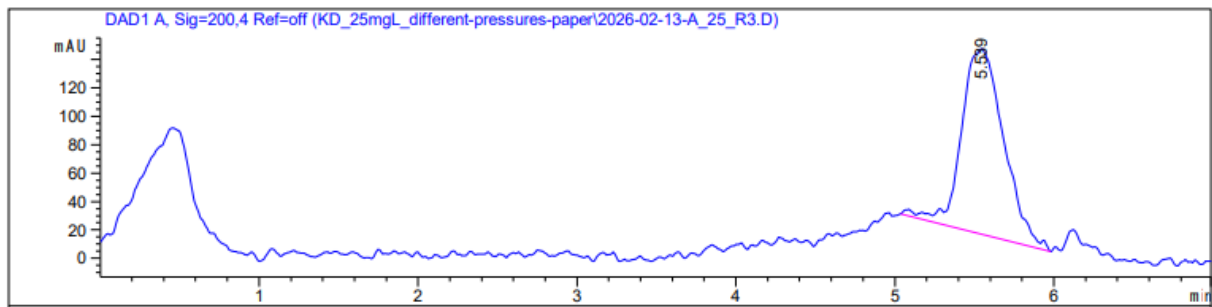
Figure S9 - HPLC chromatogram for 0.5 bar pressure – 2nd replicate.



RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [mM]	Grp	Name
5.570	BB	3669.47583	4.53170e-3	16.62896		Urea

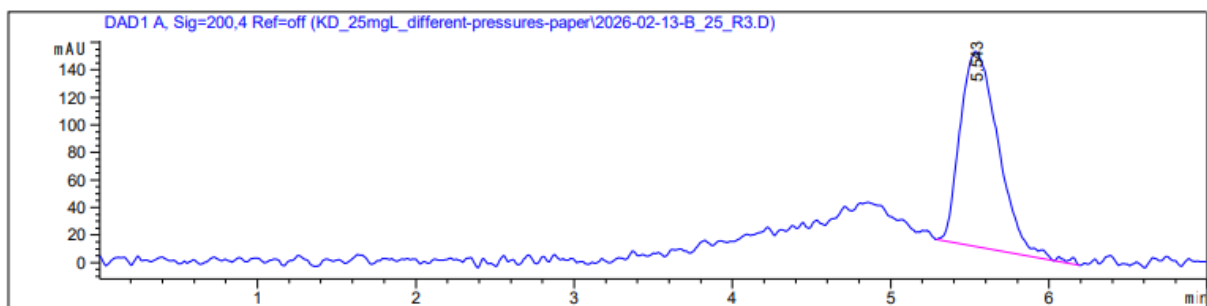
Figure S10 - HPLC chromatogram for 0.5 bar pressure – 3rd replicate.

c. 1 bar pressure in the reactor



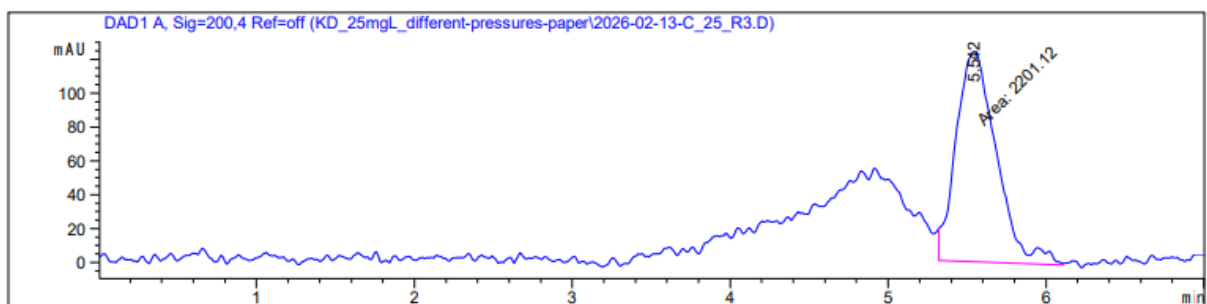
RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [mM]	Grp	Name
5.539	BB	2423.44849	4.35902e-3	10.56387		Urea

Figure S11 - HPLC chromatogram for 1 bar pressure – 1st replicate.



RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [mM]	Grp	Name
5.543	BB	2397.00610	4.35341e-3	10.43516		Urea

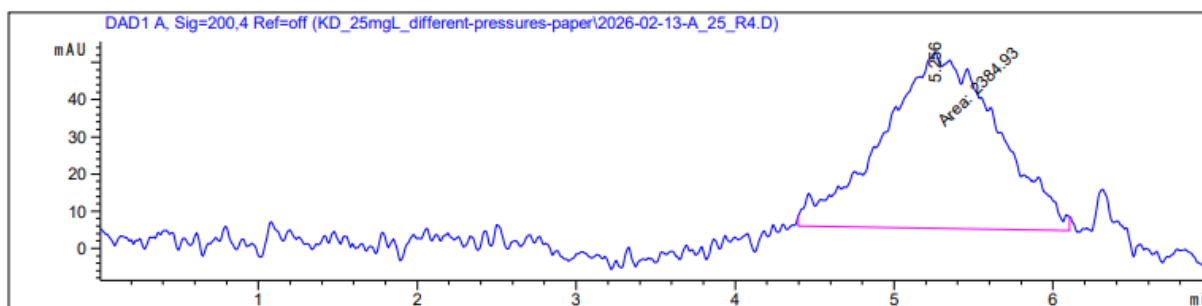
Figure S12 - HPLC chromatogram for 1 bar pressure – 2nd replicate.



RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [mM]	Grp	Name
5.542	FM	2201.12036	4.30766e-3	9.48168		Urea

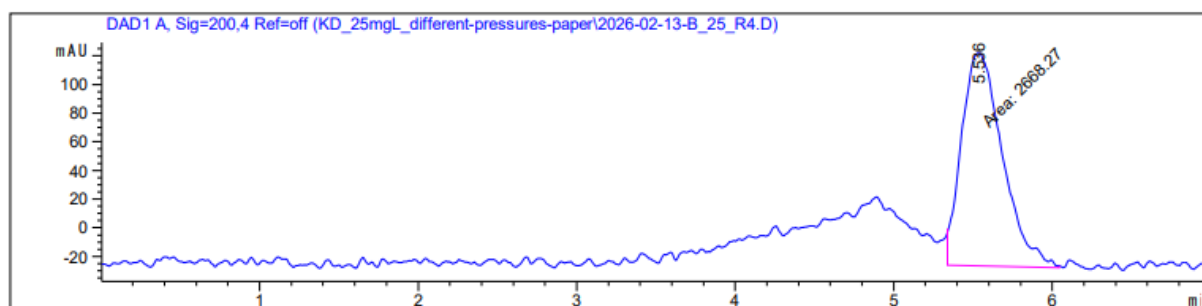
Figure S13 - HPLC chromatogram for 1 bar pressure – 3rd replicate.

d. 2.1 bar pressure



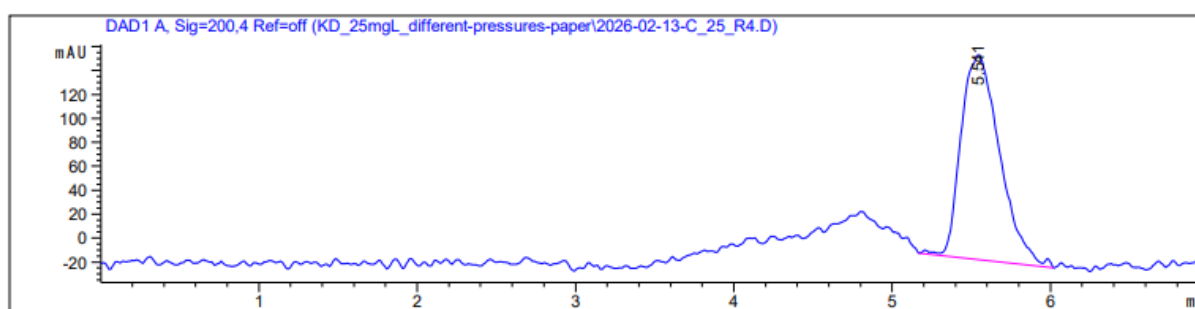
RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [mM]	Grp	Name
5.256	MM	2384.92700	4.35081e-3	10.37637		Urea

Figure S14 - HPLC chromatogram for 2.1 bar pressure – 1st replicate.



RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [mM]	Grp	Name
5.536	FM	2668.27051	4.40568e-3	11.75555		Urea

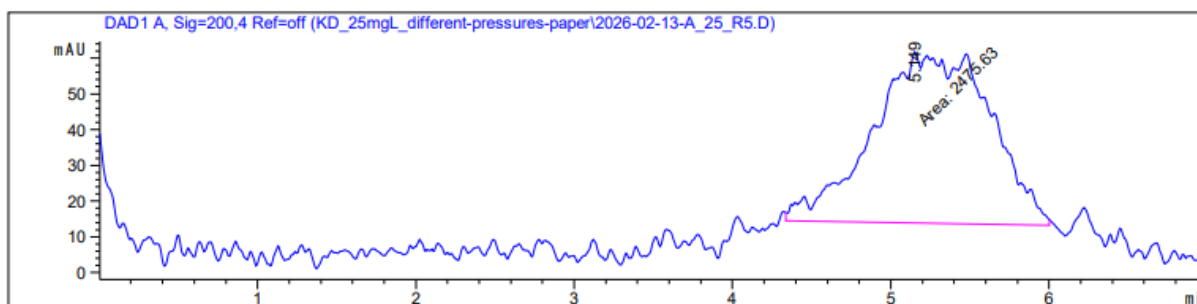
Figure S15 - HPLC chromatogram for 2.1 bar pressure – 2nd replicate.



RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [mM]	Grp	Name
5.541	BB	2909.77271	4.44401e-3	12.93107		Urea

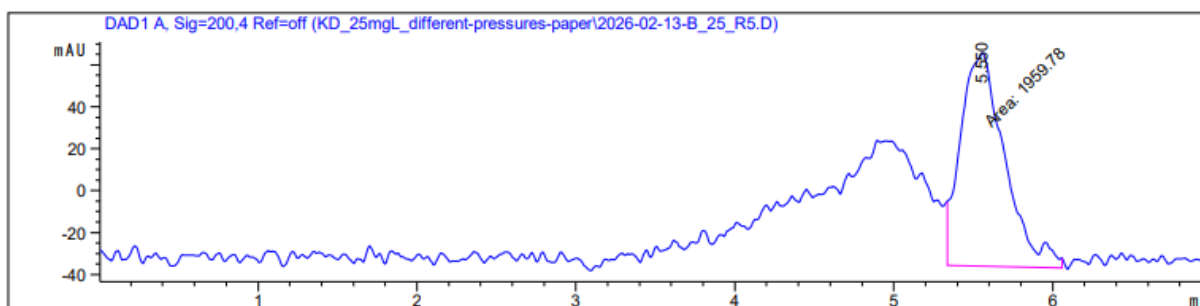
Figure S16 - HPLC chromatogram for 2.1 bar pressure – 3rd replicate.

e. 3.5 bar pressure



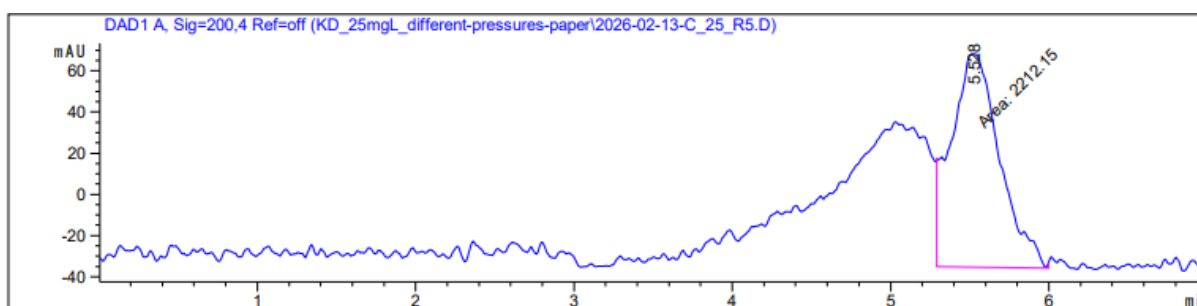
RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [mM]	Grp	Name
5.149	MM	2475.62939	4.36974e-3	10.81786		Urea

Figure S17 - HPLC chromatogram for 3.5 bar pressure – 1st replicate.



RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [mM]	Grp	Name
5.550	FM	1959.77637	4.23871e-3	8.30693		Urea

Figure S18 - HPLC chromatogram for 3.5 bar pressure – 2nd replicate.



RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [mM]	Grp	Name
5.528	FM	2212.15015	4.31045e-3	9.53537		Urea

Figure S19 - HPLC chromatogram for 3.5 bar pressure – 3rd replicate.

S7: Parameter fits for the kinetic model

The confidence ellipsoid for the parameter fit is given below.

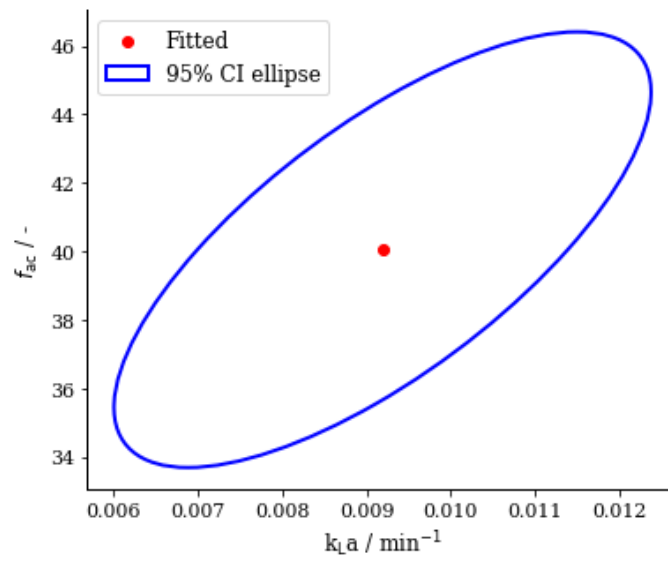


Figure S20 – Confidence ellipsoid for the parameter fit.

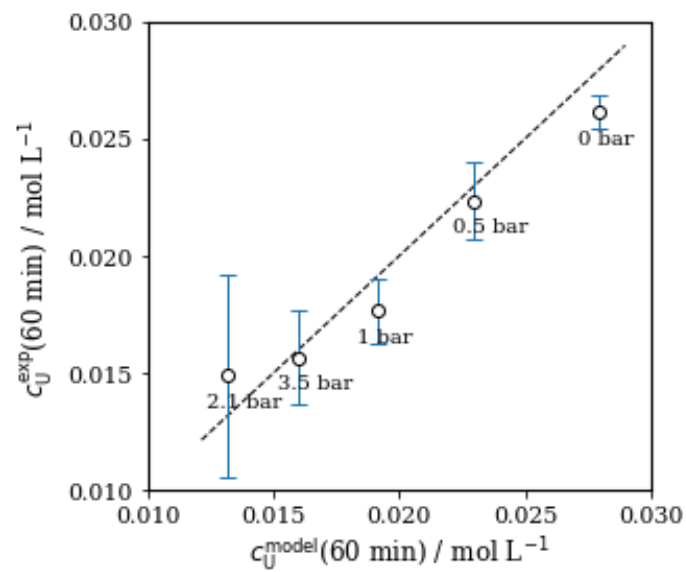


Figure S21 – Parity plot for the final urea concentrations.

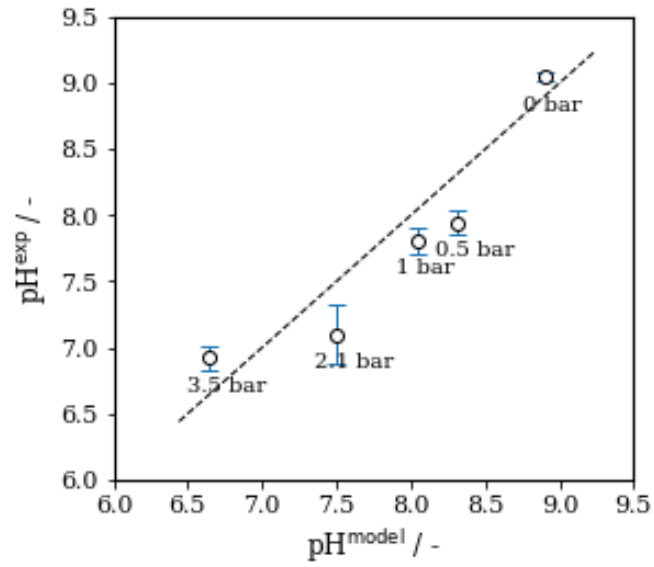


Figure S22 – Parity plot for the final pH.

S8: Results of the Bayesian optimization for the two experimentally validated points

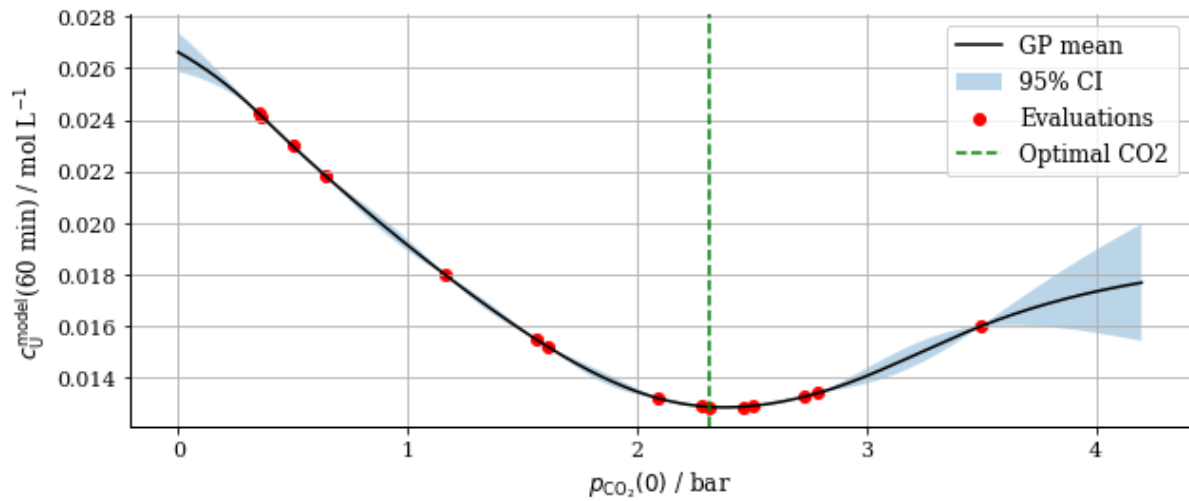


Figure S23 – Results of the Bayesian optimization for the 30 mM case.

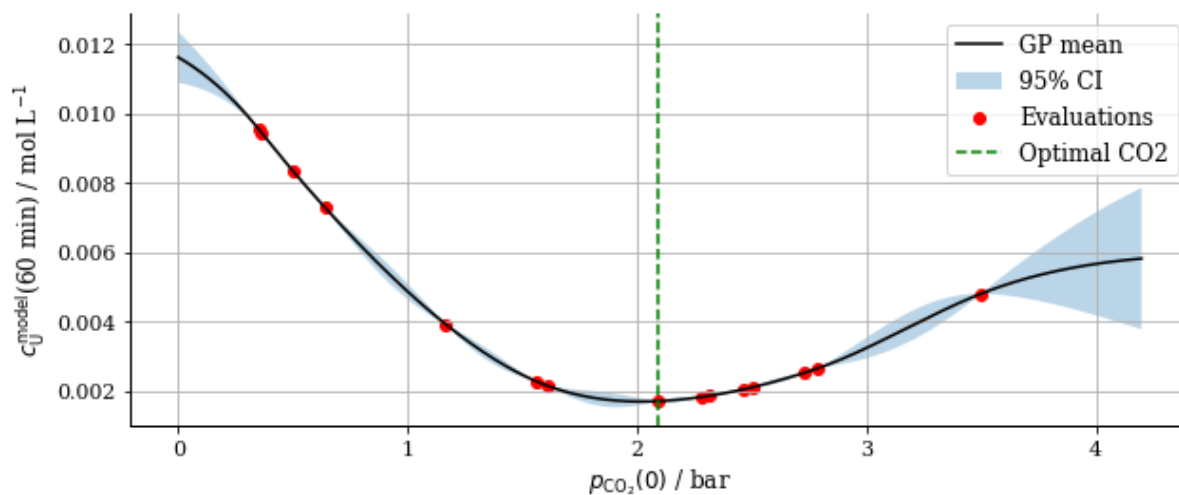


Figure S24 – Results of the Bayesian optimization for the 15 mM case.

S9: Experimental results for the two experimentally validated points

Table S7 - Experimental results for the validation of the Bayesian optimization.

Initial urea (mM)	Pressure (bar)	Final urea (mM)	pH (-)
30	2.3	9.7 ± 0.4	6.98 ± 0.15
15	2.1	4.8 ± 0.8	6.66 ± 0.12