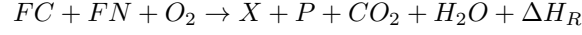


SUPPLEMENTARY MATERIAL

A. Detailed mass balances

General reaction



FN: Ammonia

FC: Glycerol

Biomass

$$Accumulation = In - Out + Generation - Consumption$$

$$\frac{d}{dt}(S \cdot V) = F_{in} \cdot S_{in,f} - F_{out} \cdot S_{out,f} - r_s \cdot X \cdot V$$

For a batch system $F_{in} = F_{out} = 0$ and V constant:

$$\frac{d}{dt}(X \cdot V) = \mu \cdot X \cdot V$$

$$\frac{dX}{dt} V = \mu \cdot X \cdot V$$

$$\frac{dX}{dt} = \mu \cdot X$$

where $\mu = r_x$ $r_x = (\mu - k_d)$ it is given by the growth rate of the organism (μ) and its dying constant (k_d)

$$\frac{dX}{dt} = (\mu - k_d) \cdot X$$

Substrates

$$Accumulation = In - Out + Generation - Consumption$$

For a batch system $F_{in} = F_{out} = 0$ and V constant:

$$Accumulation = Generation - Consumption$$

The substrate only gets consumed by the microorganism

$$\frac{d}{dt}(S \cdot V) = -r_s \cdot X \cdot V$$

where r_s is the specific consumption rate of the substrate

$$\frac{d}{dt}S = -r_s \cdot X$$

with $r_s \geq 0$

Yield

$$Y_{SX} = \left\| \frac{r_x}{r_s} \right\| = \frac{r_x}{r_s}$$

$$\frac{dS}{dt} = -\frac{\mu}{Y_{SX}} \cdot X$$

Nitrogen

$$Accumulation = In - Out + Generation - Consumption$$

$$\frac{dN}{dt} \cdot V = F_{in} - F_{out} \pm J_N \cdot V$$

For a batch system $F_{in} = F_{out} = 0$ and V constant:

$$\frac{dN}{dt} \cdot -J_N \cdot V$$

where $J_N = r_{Sp}X$ with $r_{sp} = \frac{\mu}{Y_{X/N}}$

$$\frac{dN}{dt} = -\left(\frac{\mu}{Y_{X/N}}\right) \cdot X$$

Carbon

$$\frac{dC}{dt} = -r_s \cdot X - cons$$

$$\frac{dC}{dt} = -\left(\frac{\mu}{Y_{C/X}}\right) \cdot X$$

Growth rate First the equation proposed by [?] which includes auto-inhibition factor.

$$\mu = \mu_{max} \left(1 - e^{\frac{-t}{\tau_{lag}}}\right) \left(\frac{C}{C + k_C}\right) \left(\frac{N}{N + k_N}\right) \left(1 - \frac{X}{X_{max}}\right)$$

Then, for considering the influence of pH in the growth behavior the multiplication of a I_{pH} factor proposed by [?] is included.

$$I_{pH} = e^{\left(I_{val} \frac{pH - pH_{UL}}{pH_{UL} - pH_{LL}}\right)^2}$$

Then the growth rate ends up as:

$$\mu = \mu_{max} \left(1 - e^{\frac{-t}{\tau_{lag}}}\right) \left(\frac{C}{C + k_C}\right) \left(\frac{N}{N + k_N}\right) \left(1 - \frac{X}{X_{max}}\right) \cdot I_{pH}$$

B. Detailed ion concentration equations

The dissociation equations considered can be seen below:

TABLE S1: pKa values and acid dissociation equations of media components

Constituent	pK_a	Acid Dissociation Equations
KH_2PO_4	6.86 (pKa1)	$KH_2PO_4 \leftrightarrow H^+ + KHPO_4^-$
Citric acid	3.13 (pKa2)	$C_6H_8O_7 \leftrightarrow H^+ + C_6H_7O_7^-$
	4.76 (pKa3)	$C_6H_7O_7^- \leftrightarrow H^+ + C_6H_6O_7^{2-}$
	6.40 (pKa4)	$C_6H_6O_7^{2-} \leftrightarrow H^+ + C_6H_5O_7^{3-}$
CO_2	6.35 (pKa5)	$CO_2 + H_2O \leftrightarrow H^+ + HCO_3^-$
H_2O	14 (pKa6)	$H_2O \leftrightarrow H^+ + OH^-$

Obtained from [1], considering normal conditions of T and P.

Firstly, it is explained how the term I_c is defined. In order to balance the charges, we can consider the following, with the species mentioned:

$$[H^+] + 2[Mg^{2+}] + [Na^+] + 2[Zn^{2+}] = [OH^-] + [KHPO_4^-] + 3[C_6H_5O_7^{3-}] + 2[C_6H_6O_7^{2-}] + [C_6H_7O_7^-] + [HCO_3^-] + [SO_4^{2-}]$$

Those defined as constant throughout time, $[Mg^{2+}]$, $[Na^+]$, $[Zn^{2+}]$ and $[SO_4^{2-}]$ are grouped in a single term for simplicity,

$$[I_c] = [SO_4^{2-}] - [Mg^{2+}] - [Na^+] - [Zn^{2+}]$$

Therefore, yielding the balance proposed above.

Secondly, in a generic Henderson-Hasselbach acid-base dissociation reaction as shown in equation below, with HA being an acid, we can establish the acidic equilibrium constant K_a apyotesis.

$$HA \rightleftharpoons H^+ + A^-$$

$$K_a = \frac{[H^+][A^-]}{[HA]}$$

The total concentration of the acid in the culture corresponds to the concentration of its anions plus the non-dissociated amount,

$$[HA_{total}] = [A^-] + [HA]$$

Using both equations, we get to the following expression,

$$[HA_{total}] = [A^-] + \frac{[H^+][A^-]}{[K_a]}$$

In terms of the acid anion, then we have,

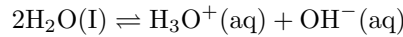
$$[A^-] = \frac{[HA_{total}]}{\left(1 + \frac{[H^+]}{K_a}\right)}$$

This resembles Hill's equation, showing the probability of the acid to be in its dissociated form.

Now, this procedure can be done for the reactions presented in Table S1. For example, KH_2PO_4 :

$$[KHPO_4^-] = \frac{KH_2PO_{4, \text{ tot}}}{\left(1 + \frac{[H^+]}{K_{a1}}\right)}$$

For H_2O it was considered a different approach. This was the standard water autoionization, corresponding to,



$$K_{aw} = [H^+][OH^-]$$

$$[OH^-] = \frac{K_w}{[H^+]}$$

C. Constitutive equations: Charge balance

The ionic species observed in Table S1 are balanced in an charge balance. All H^+ ions were consolidated into a single variable H^+ , allowing for the determination of its concentration based on the concentrations of other ionic species in the culture. This balance is illustrated in Equation 1.

$$[H^+] = [OH^-] + [KHPO_4^-] + 3[C_6H_5O_7^{3-}] + 2[C_6H_6O_7^{2-}] + [C_6H_7O_7^-] + [HCO_3^-] - [I_c] \quad (1)$$

To solve the equation and determine the concentration of H^+ at any given moment, an expression was derived for each ionic compound. The definition for each item was established on the principles of acid-base equilibrium, and detailed derivations can be found in Appendix -B. Particularly, equations 2, 3, 4, 5 and 6 only have the H^+ as a variable that changes overtime, while 7 has not only the proton term, but also CO_2 dependence, a variable defined for the growth dynamic above.

$$[OH^-] = \frac{K_{a6}}{[H^+]} \quad (2)$$

$$[KHPO_4^-] = \frac{KH_2PO_{4, \text{ tot}}}{\left(1 + \frac{[H^+]}{K_{a1}}\right)} \quad (3)$$

$$[C_6H_5O_7^{3-}] = \frac{C_6H_8O_7, \text{ tot}}{\frac{[H^+]^3}{K_{a2}K_{a3}K_{a4}} + \frac{[H^+]^2}{K_{a3}K_{a4}} + \frac{[H^+]}{K_{a4}} + 1} \quad (4)$$

$$[C_6H_6O_7^{2-}] = \frac{[H^+] \cdot [C_6H_5O_7^{3-}]}{K_{a4}} \quad (5)$$

$$[C_6H_7O_7^-] = \frac{[H^+] \cdot [C_6H_6O_7^{2-}]}{K_{a3}} \quad (6)$$

$$[HCO_3^-] = \frac{CO_{2, \text{ tot}}}{\left(\frac{[H^+]}{K_{a5}} + 1\right)} \quad (7)$$

D. System Conditions and Data

TABLE S2: Model Parameters Used in the Dynamic Simulation of *M. smegmatis* Growth

Parameter	Description	Value
t_{lag}	Lag phase duration [h]	7
k_C	Monod constant for glycerol [g/L]	0.3828
k_N	Monod constant for ammonia [g/L]	1.0027×10^{-4}
$Y_{X/C}$	Biomass yield on glycerol [g/g]	0.484
$Y_{X/N}$	Biomass yield on ammonia [g/g]	21.575
Y_{X/CO_2}	CO ₂ yield [g/g]	0.3687
Y_{X/O_2}	O ₂ yield [g/g]	1.352
X_{max}	Maximum biomass concentration [g/L]	1.4462
μ_{max}	Maximum specific growth rate [1/h]	0.19
k_d	Death rate constant [1/h]	0.001
pH _{LL}	Lower limit of pH tolerance [-]	3.4687
pH _{UL}	Upper limit of pH tolerance [-]	7.4
I_{val}	pH inhibition shape parameter [-]	4.0413
$O_{2,\text{sat}}$	O ₂ saturation concentration [g/L]	7.267×10^{-3}
k_{La}	Oxygen transfer coefficient [1/h]	86.26
k_O	Monod constant for oxygen [g/L]	4.5×10^{-3}

TABLE S3: Model Constants

Parameter	Chemical species or group	Value
pK_{a1}	KH ₂ PO ₄ (phosphate buffer)	6.86
pK_{a2}	Citric acid (C ₆ H ₈ O ₇)	3.13
pK_{a3}	Mono-deprotonated citric acid (C ₆ H ₇ O ₇) ⁻	4.76
pK_{a4}	Di-deprotonated citric acid (C ₆ H ₇ O ₇) ²⁻	6.40
pK_{a5}	Ammonium (NH ₃)	9.25
pK_{a6}	Glycerol (C ₃ H ₈ O ₃)	14.15
pK_{a7}	Carbon dioxide (CO ₂)	6.35
pK_{a8}	Bicarbonate (HCO ₃ ⁻)	10.33
pK_{a9}	Water (H ₂ O)	14.00
[KH ₂ PO ₄]	Phosphate buffer (monobasic) [mol/L]	2.18
[C ₆ H ₈ O ₇]	Citric acid (tricarboxylic acid) [mol/L]	2.00
pH _{alk}	Constant ions in pH charge balance [-]	7.2

TABLE S4: Initial Conditions Used in the Simulation of *M. smegmatis* Growth

Variable	Description	Parameter Estimation	Validation
X_0	Biomass concentration [g/L]	0.229	0.223
C_0	Glycerol concentration [g/L]	5.389	5.922
N_0	Ammonium concentration [g/L]	0.951	1.027
$CO_{2,0}$	Carbon dioxide concentration [g/L]	4.39×10^{-4}	4.39×10^{-4}
$O_{2,0}$	Dissolved oxygen concentration [g/L]	0.0001	0.0001

E. Model Comparison

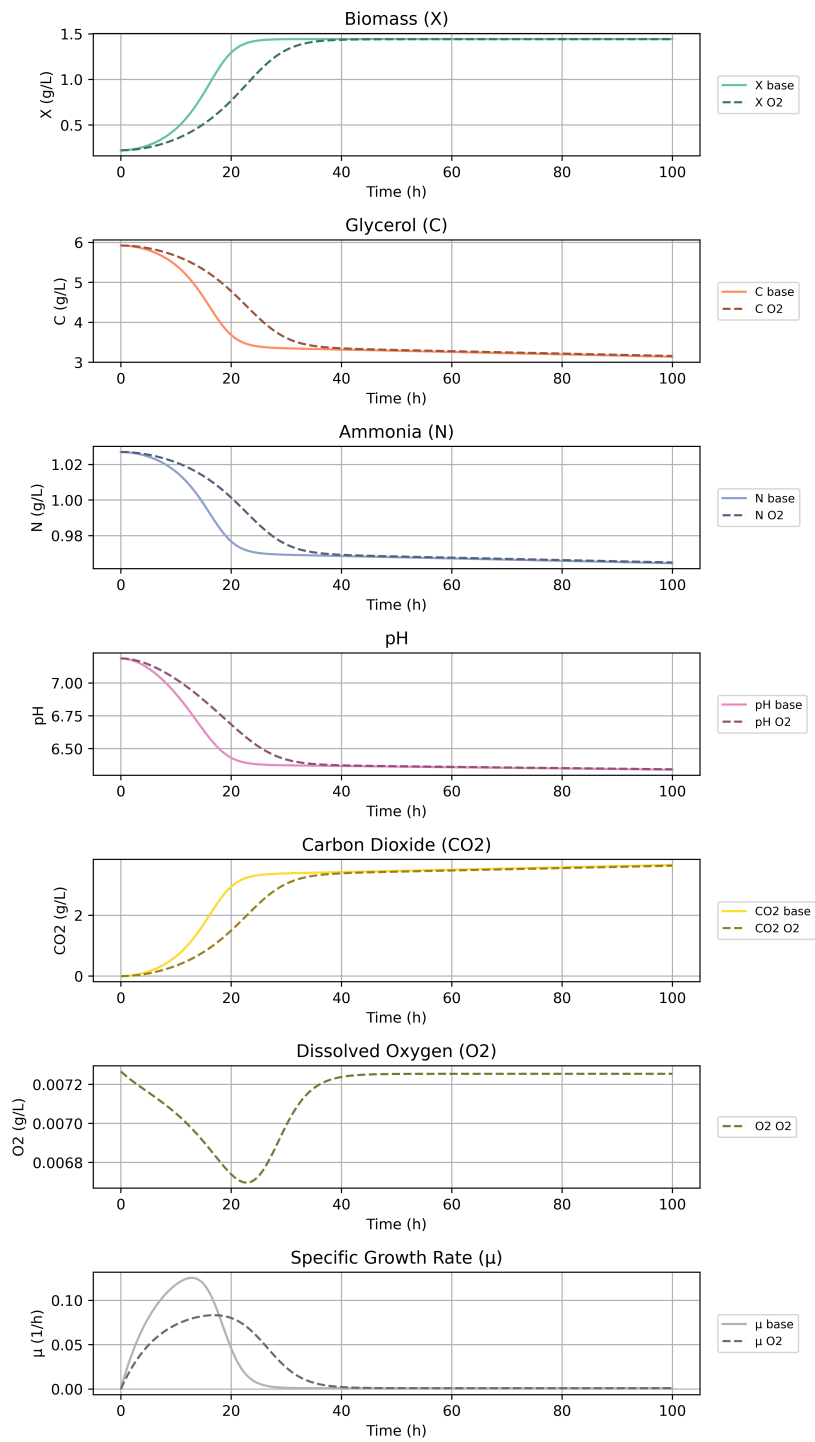


Fig. S1: Comparison between original model without oxygen, and the updated model with oxygen consumption on hypoxic conditions.

F. Original Model Validation Results

k_C : 0.08
 k_N : 0.01
 k_O : 0.001
 k_d : 0.001
 YX_{CO2} : 0.35
 YX_{O2} : 1.352
 YX_C : 0.484
 YX_N : 21.575
 X_{max} : 1.4462
 μ_{max} : 0.15
 pH_{LL} : 4.6
 pH_{UL} : 7.4
 I_{val} : 3
 $O2_{sat}$: 0.007267
 t_{lag} : 7
 k_{La} : 86.26

AIC = -110.18

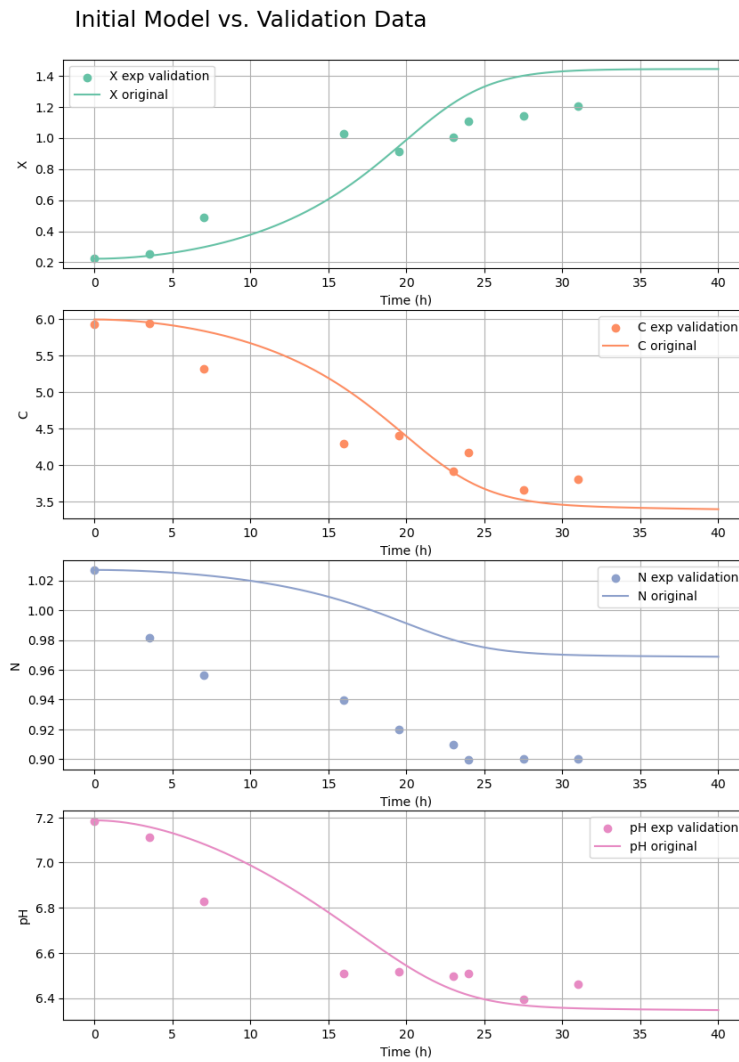


Fig. S2: Validation Experiment Points vs Original Model

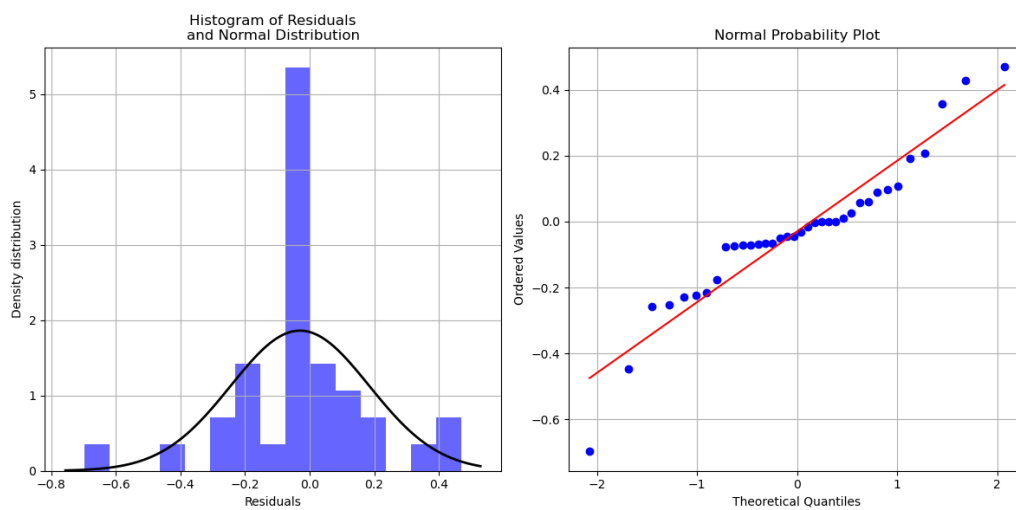


Fig. S3: Normal Distribution Fitting and Normal Probability Plot for Original Model

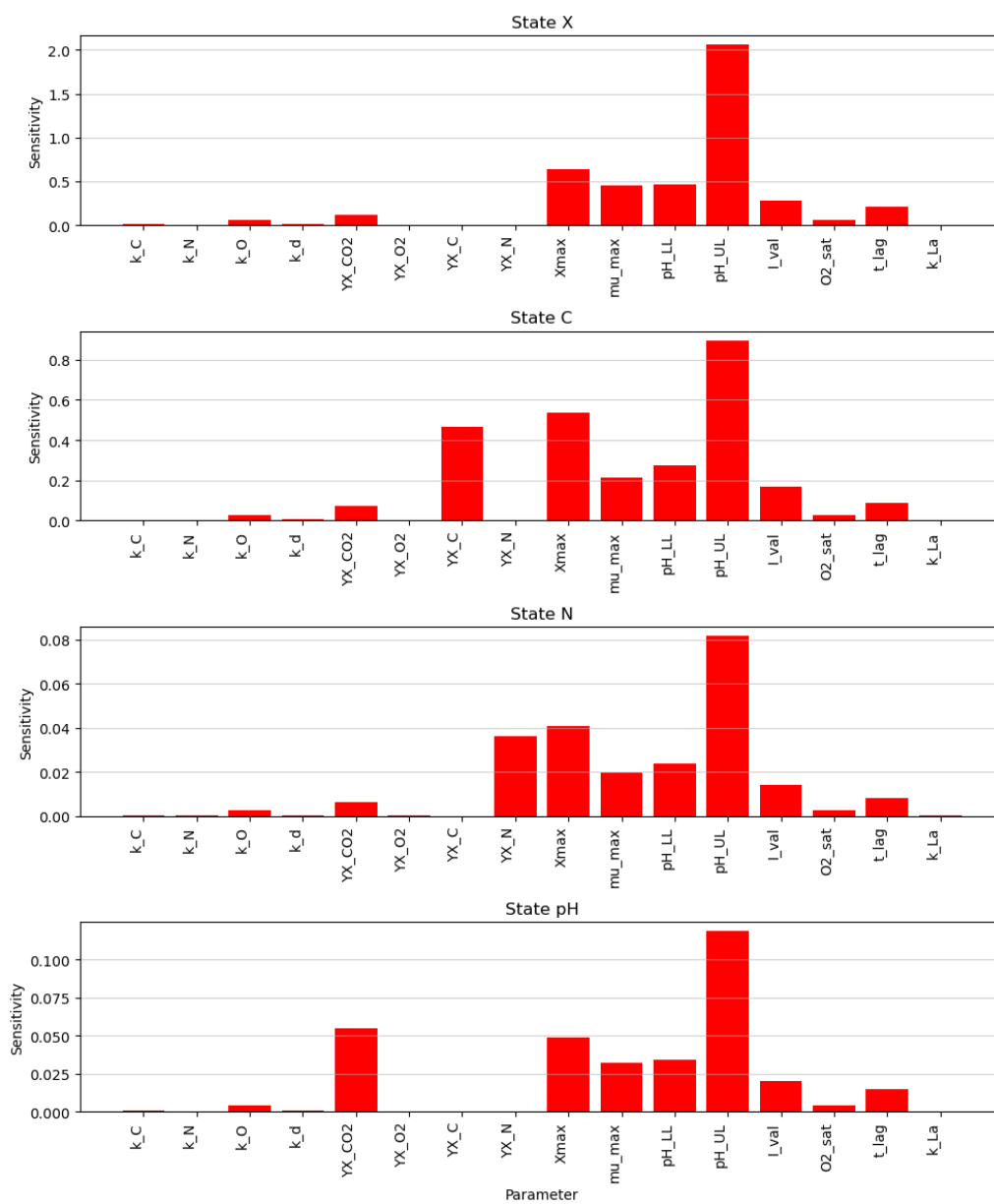


Fig. S4: Sensitivity Analysis of Parameters for Original Model

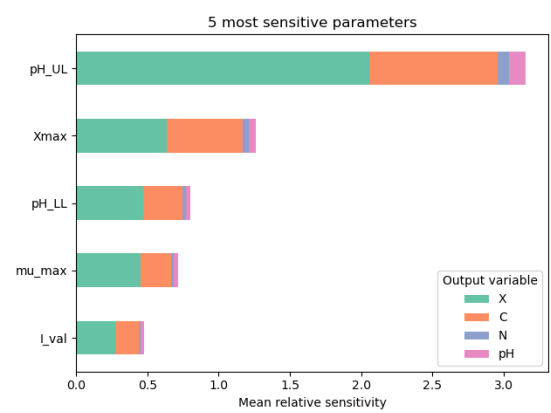


Fig. S5: Most Sensitive Parameters of Parameters for Original Model

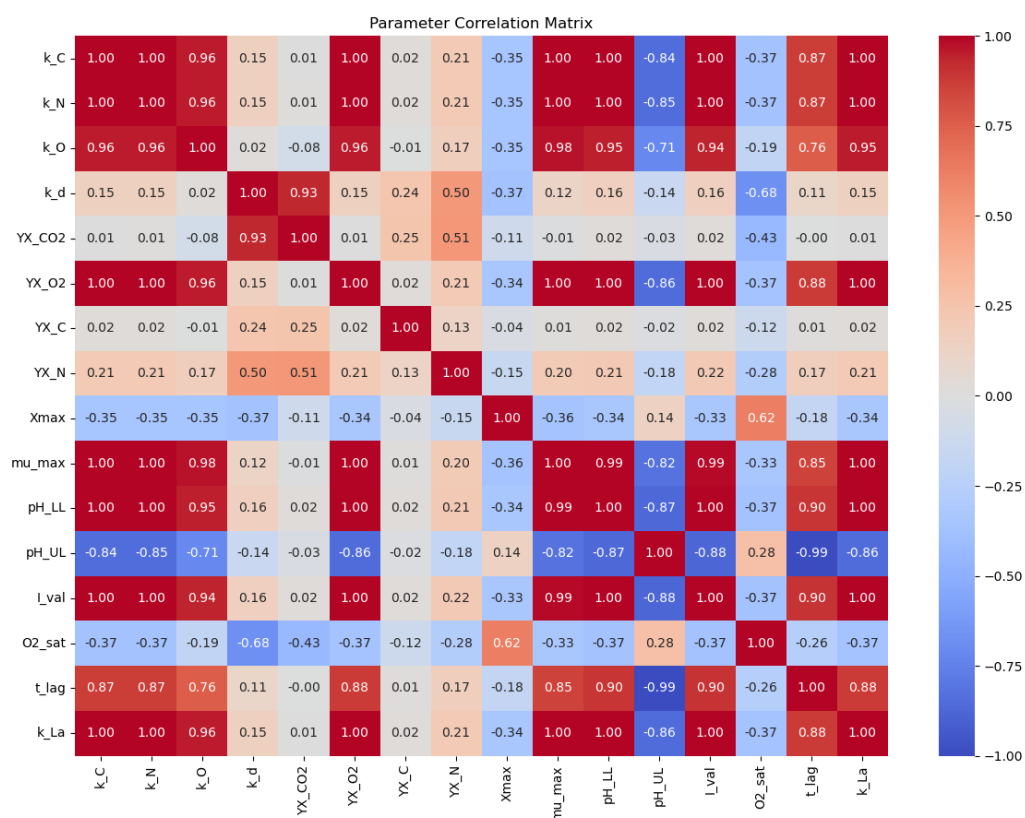


Fig. S6: Correlation Matrix for Original Model

G. Parameter Estimation Results

1) *Estimating X_{max} and $Y_{X/N}$* : $Y_{X/N}$ has a high initial value; therefore, smaller values were used to lower its stationary phase simulation. It was found that a value of allows for a better approximation of stationary phase. Lowering X_{max} value also allows for Biomass better stationary phase. Iterations were performed, and the best results were obtained with 5 and 8 for lower and upper bound for Y_{XN} and 1.1 and 1.3 for X_{max} . The final iteration, considered the best one, can be seen compared to experimental data next.

New Model fitting [' $Y_{X/N}$ ', ' X_{max} '] vs. Validation Data

k_C : 0.08
 k_N : 0.01
 k_O : 0.001
 k_d : 0.001
 $Y_{X_{CO2}}$: 0.35
 $Y_{X_{O2}}$: 1.352
 Y_{X_C} : 0.484
 Y_{X_N} : 7.999999999999922
 X_{max} : 1.182930520242307
 μ_{max} : 0.15
 pH_{LL} : 4.6
 pH_{UL} : 7.4
 I_{val} : 3
 $O2_{sat}$: 0.007267
 t_{lag} : 7
 k_{La} : 86.26

AIC = -106.36

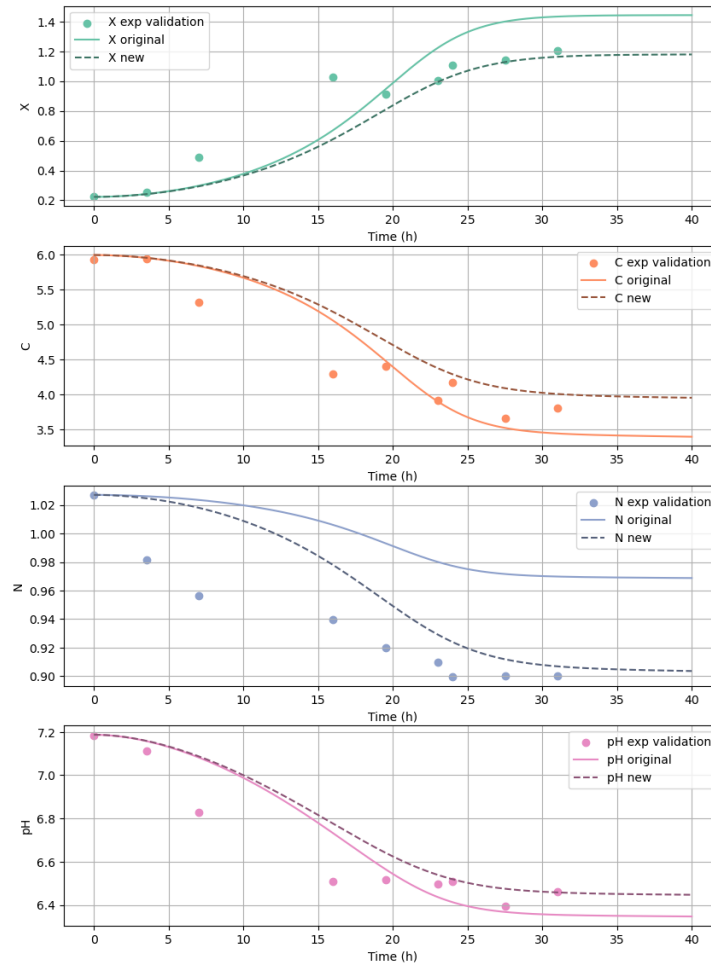


Fig. S7: Validation Experiment Points vs First Calibrated Model

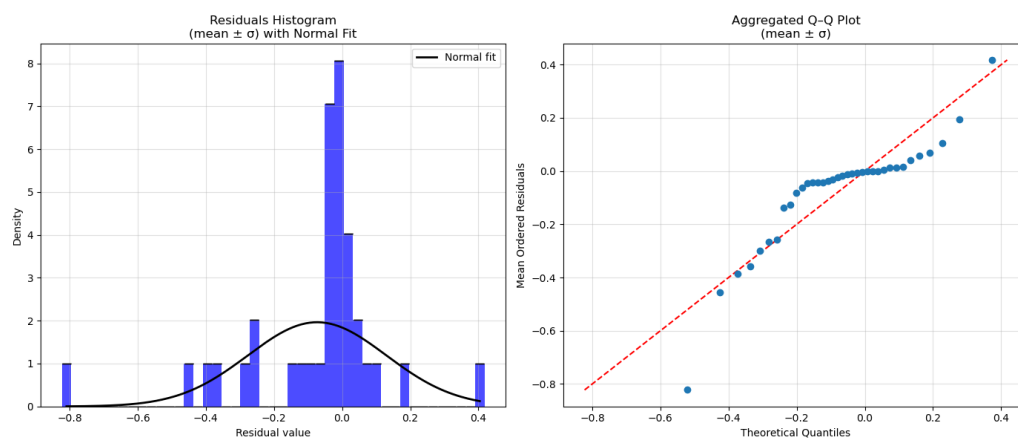


Fig. S8: Normal Distribution Fitting and Normal Probability Plot for First Calibration

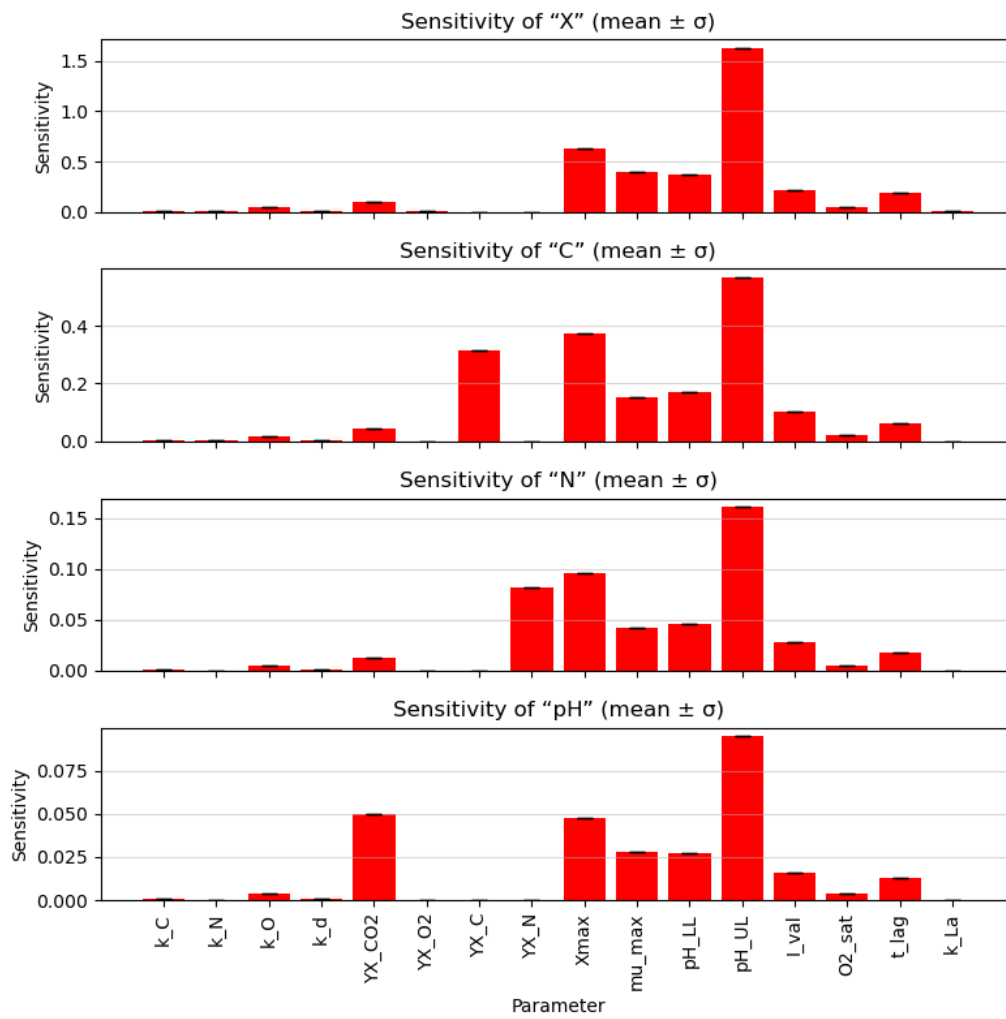


Fig. S9: Sensitivity Analysis of Parameters for First Calibrated Model

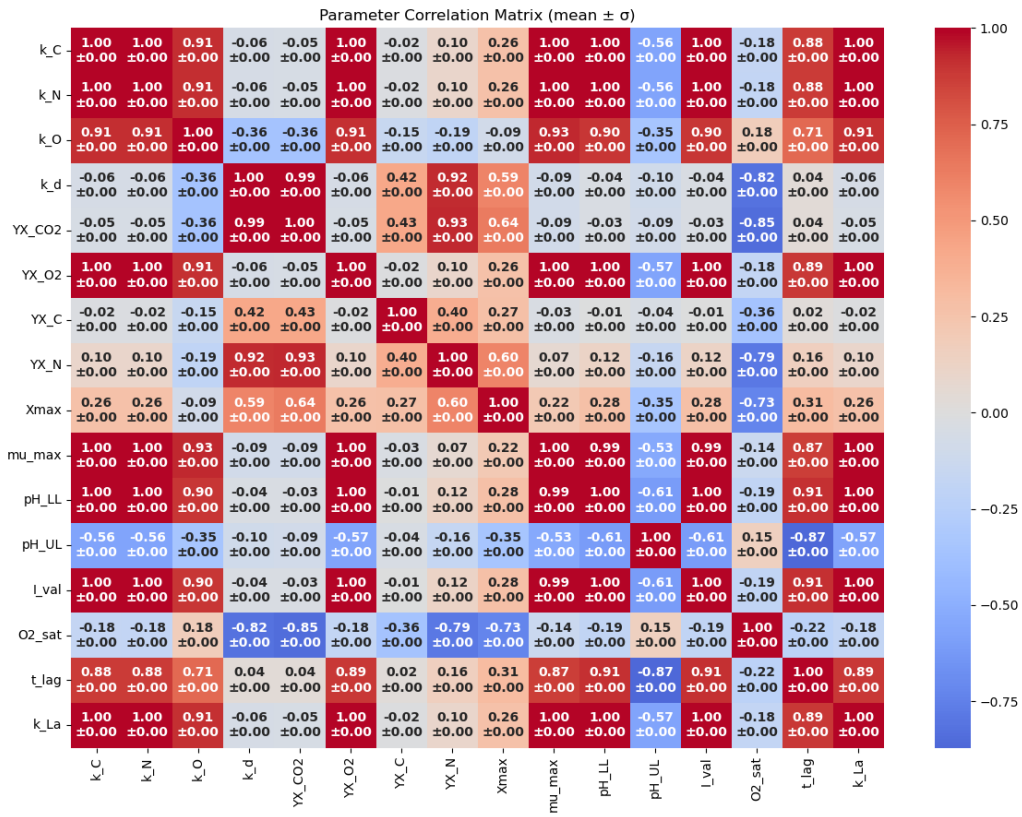


Fig. S10: Correlation Matrix for First Calibrated Model

Computed t-values for calibrated parameters:

Parameter	θ	SE	t-value
YX_N	8.000000	0.006930	1154.42
Xmax	1.182931	0.000099	11899.58

Fig. S11: t values for First Calibrated Parameters

2) Estimating pH_{UL} and μ_{max} : Estimating pH_{UL} around a neutral value, and μ_{max} to a higher value better AIC is obtained. Many iterations were performed until the best AIC values were obtained. The bound used for the best model were 6 and 7.

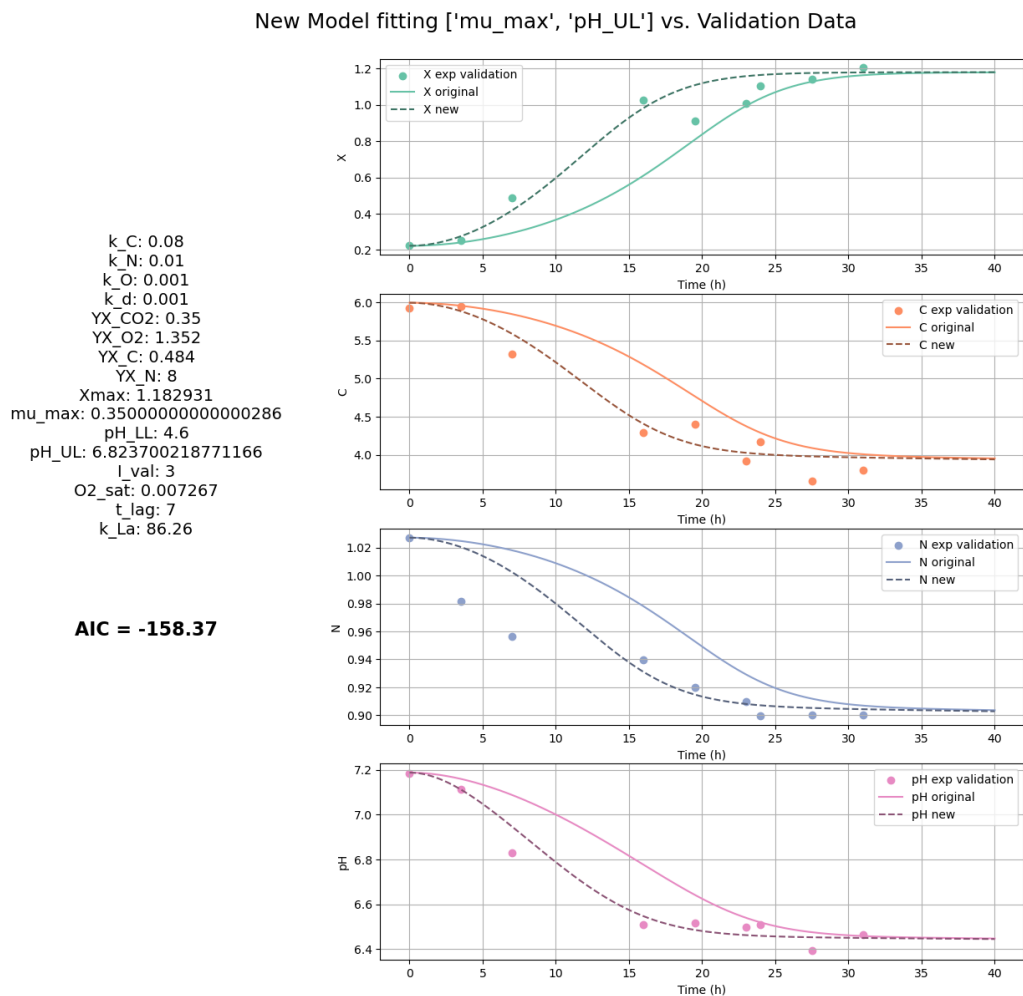


Fig. S12: Validation Experiment Points vs Second Calibrated Model

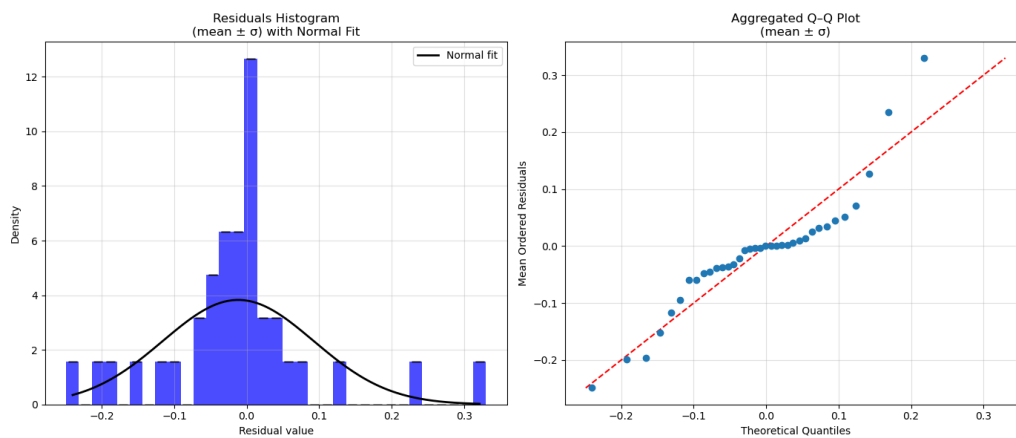


Fig. S13: Normal Distribution Fitting and Normal Probability Plot for Second calibration

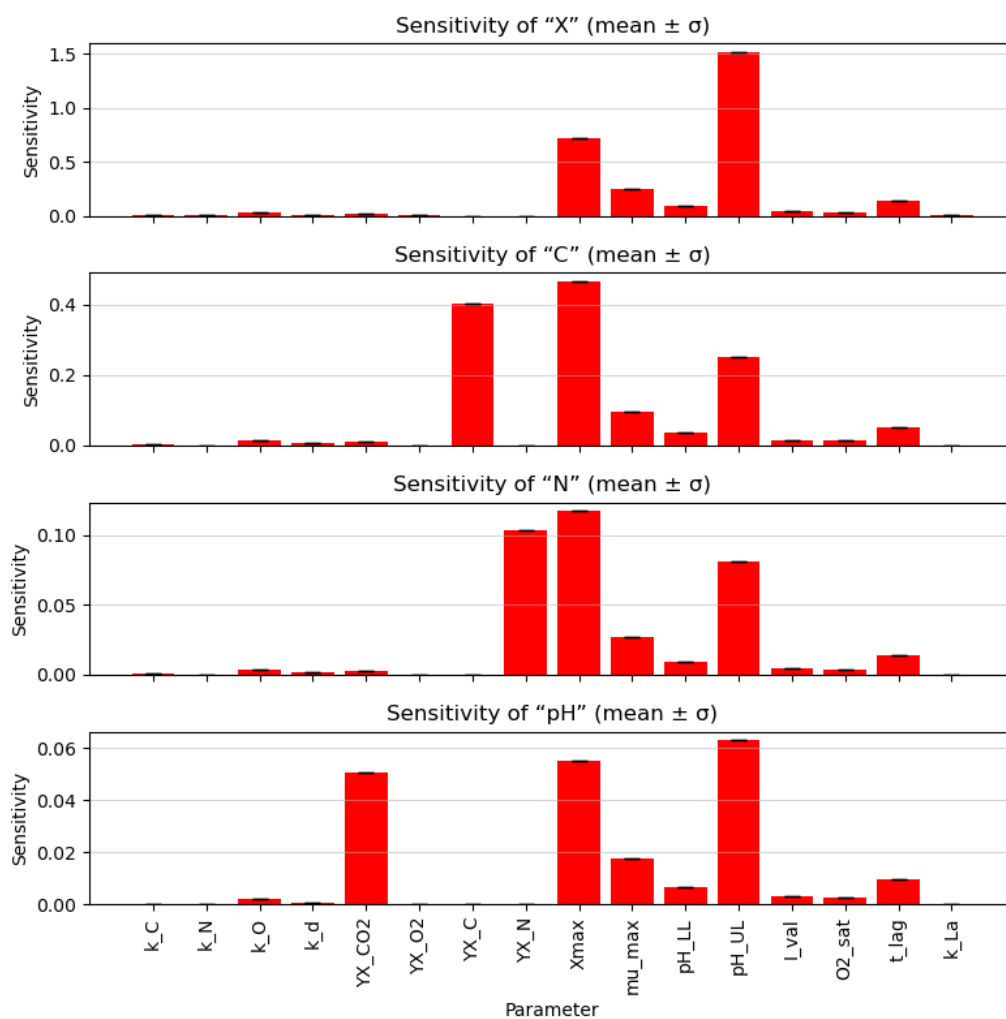


Fig. S14: Sensitivity Analysis of Parameters for Second Calibrated Model

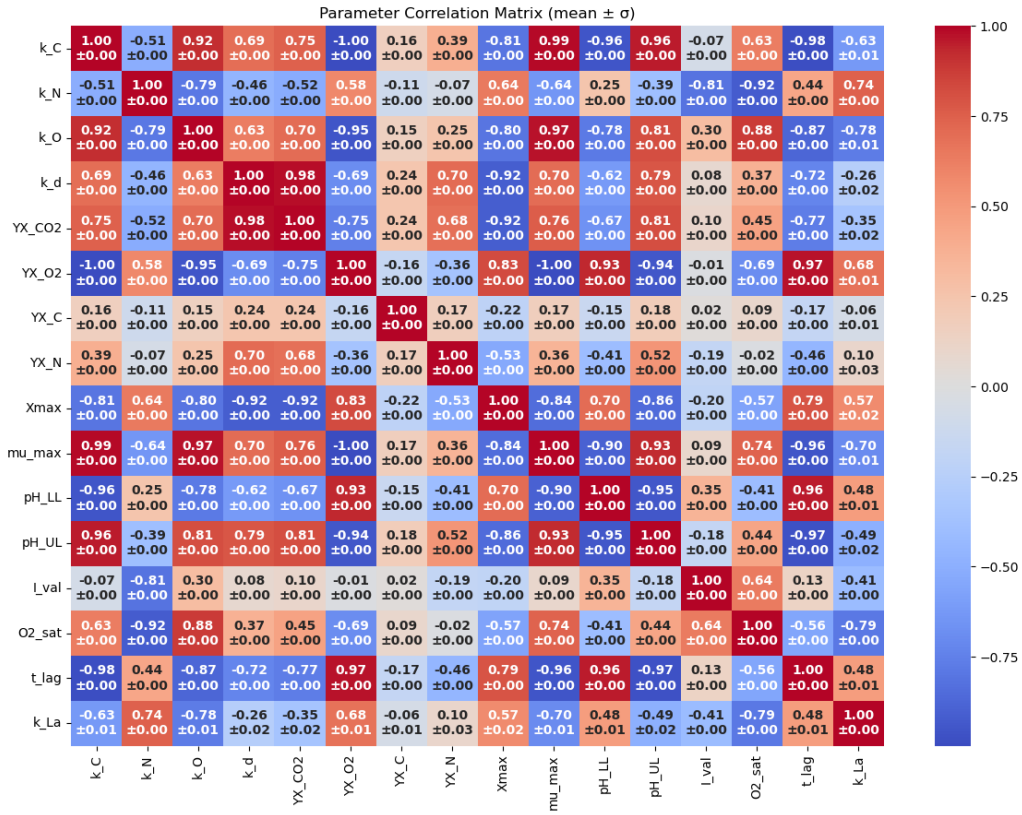


Fig. S15: Correlation Matrix for Second Calibrated Model

REFERENCES

- [1] Lide, D. and Frederikse, H. *CRC Handbook of Chemistry and Physics: A Ready-Reference Book of Chemical and Physical Data*, CRC Press, Boca Raton, FL, 1993.