Sensitivity-Driven Optimization of a Batch Cultivation DAE Model of *Mycobacterium smegmatis*

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***Abstract*—Tuberculosis remains a leading cause of mortality worldwide, driven by the adaptive metabolism of *Mycobacterium tuberculosis*. Experimental studies using the non-pathogenic surrogate *Mycobacterium smegmatis* are often costly and time-consuming. To accelerate *in silico* investigations of mycobacterial metabolism, a previously developed dynamic model based on differential–algebraic equations was refined by explicitly incorporating oxygen transfer and consumption dynamics. A CasADi-based Python package, `DAE-Model-Calibration-and-Analysis`, was developed to automate DAE formulation, simulation, sensitivity analysis, and parameter calibration workflows. By applying a sensitivity analysis, the original 26 model fitting parameters were reduced to 4. Hence, the Akaike Information Criterion was reduced by more than 40% and the normalized fitting errors for biomass, glycerol, ammonia, and pH to single-digit values. Future work will focus on enhancing the package's optimization robustness, integrating additional algorithms to expand its functionality, and validating model performance under further stress conditions.**

***Index Terms*—Differential algebraic equations, Mycobacterium smegmatis, oxygen dynamics, model calibration, CasADi, Particle Swarm Optimization, sensitivity analysis, Tuberculosis**

I. Introduction

Tuberculosis (TB) remains the leading cause of death from infectious diseases worldwide [1]. In 2023, an estimated 10.8 million people developed TB, resulting in approximately 1.25 million deaths [1]. To meet the 2027 targets set by the United Nations, an annual investment of USD 22 billion is required for prevention, diagnosis, treatment, and care [1].

TB is caused by *Mycobacterium tuberculosis*, a pathogen capable of persisting in a dormant state within the host [2]. During latency, the bacterium undergoes physiological adaptations that confer phenotypic drug resistance, making it difficult to eradicate [2]. As part of the immune response, macrophages phagocytose the bacilli and initiate granuloma formation to contain the infection [2]. Within granulomas, *M. tuberculosis* experiences carbon starvation, oxygen limitation, and acidic pH, which further drive adaptive responses [2].

Because direct *in vitro* work with *M. tuberculosis* is hazardous, researchers often employ the non-pathogenic surrogate *Mycobacterium smegmatis*. This fast-growing species shares key metabolic and regulatory traits with *M. tuberculosis*, making it a cost-effective model for studying mycobacterial physiology [3]. Nevertheless, wet-lab experiments are labor-intensive and limited in scalability [4].

Computational modeling provides a flexible alternative for exploring bacterial behavior under controlled conditions, integrating diverse datasets and performing *in silico* experiments that complement laboratory research [4].Many approaches—ordinary and partial differential equations, agent-based models and hybrid multiscale frameworks—have been used to investigate immune responses, granuloma formation, cytokine signaling and nutrient dynamics during TB infection [4].

Focusing on latency, Magombedze and Mulder [5] developed a gene-expression–based ODE model of *M. tuberculosis* under nitric oxide, hypoxia, and nutrient deprivation, reproduc-

ing transitions between replicative and non-replicative states. Ibargüen-Mondragón et al. [6] constructed an ODE model of macrophage–T cell–bacteria interactions, revealing multiple infection equilibria and bistable responses driven by immune clearance rates and bacterial competition.

Unlike host–pathogen or population-level models, this work examines the metabolic dynamics of *M. smegmatis* in batch culture. Building on a previously published DAE framework [7], the model was refined by explicitly incorporating oxygen transfer and consumption. Additionally, a CasADi-based Python package was developed to automate and simplify DAE formulation, simulation, sensitivity analysis, and parameter calibration—which are time-consuming. Local sensitivity analysis and Fisher Information Matrix evaluation guided the selection of parameters for calibration, yielding a model with improved fitting accuracy.

II. Methodology

A. Model Extension and Implementation

This work extends the dynamic model proposed in [8], which itself derives from the foundational model proposed in [7]. The present formulation preserves the following elements from [7]: it is based on Monod kinetics, considering glycerol and ammonium as limiting substrates; it introduces additional terms to improve predictive accuracy, one such term accounts for the inhibition of bacterial growth due to medium acidification, while another models the lag phase. Biomass production, substrate consumption, and metabolite formation are all described through systems of ordinary differential equations (ODEs). Furthermore, a pH estimation model is incorporated by solving an electrical charge balance, accounting for dissociated species in the medium.

In the extension developed by [8], the carbon dioxide (CO_2) production equation is modified to capture its role in pH regulation, and an additional term is introduced into the growth rate expression to represent bacterial self-inhibition at high biomass concentrations.

The model proposed in this work further expands the previous frameworks by incorporating the oxygen dynamics described by [7], including an ODE for oxygen concentration and its integration into the growth rate expression.

B. Mathematical modeling

1) Mass balances: The biomass concentration X (g/L) was modeled through a net growth rate that accounts for microbial proliferation and decay, where μ is the specific growth rate (1/h) and k_d is the constant specific death rate (1/h):

$$\frac{dX}{dt} = (\mu - k_d) \cdot X \tag{1}$$

The glycerol concentration C (g/L) was modeled based on its consumption for biomass formation, assuming Monod-type kinetics. The rate of consumption is proportional to X and μ, scaled by the yield coefficient $Y_{X/C}$ (g biomass/g glycerol):

$$\frac{dC}{dt} = -\left(\frac{\mu}{Y_{X/C}}\right) \cdot X \tag{2}$$

The ammonium concentration N (g/L) was modeled analogously to the carbon source, with consumption driven by biomass growth and scaled by the yield coefficient $Y_{X/N}$ (g biomass/g ammonium):

$$\frac{dN}{dt} = -\left(\frac{\mu}{Y_{X/N}}\right) \cdot X \tag{3}$$

Carbon dioxide concentration CO_2 (g/L) was modeled as a product of biomass growth, scaled by the yield coefficient Y_{X/CO_2} (g biomass/g CO_2). Additionally, the model includes its consumption through the formation of bicarbonate, which depends on the proton concentration $[H^+]$ and contributes to pH regulation. The parameter K_{a5} (mol/L) is an equilibrium constant that represents the dissociation of carbonic acid according to acid–base reactions:

$$\frac{dCO_2}{dt} = \frac{\mu}{Y_{X/CO_2}} \cdot X - K_{a5} \cdot \frac{CO_2}{\frac{[H^+]}{K_{a5}} + 1} \tag{4}$$

The dynamics of dissolved oxygen concentration O_2 (g/L) were modeled through a differential equation that accounts for two key processes: gas-liquid transfer and microbial consumption. The transfer is described by a term driven by the difference between the saturation concentration $O_{2,\text{sat}}$ (g/L) and the actual dissolved oxygen, scaled by the volumetric oxygen transfer coefficient k_La (1/h). The consumption term reflects oxygen uptake due to biomass growth, governed by μ, X, and the biomass yield per unit of oxygen consumed Y_{X/O_2} (g biomass/g O_2). This dynamic was not included in the previously proposed models, since no oxygen limitation was observed under their experimental conditions. However, oxygen availability is known to play a critical role in systems involving biofilms and granulomas, where diffusion limitations become relevant [7]. Therefore, explicit inclusion of oxygen dynamics is essential to improve the physiological realism and predictive capacity of the model in such contexts.

$$\frac{dO_2}{dt} = k_La \cdot (O_{2,\text{sat}} - O_2) - \frac{\mu}{Y_{X/O_2}} \cdot X \tag{5}$$

The derivation and mass balances for these equations can be found in the Supplementary Material Section A.

2) Charge balance: The pH of the growth medium was modeled dynamically through an electro-neutrality condition that accounts for the dissociation of relevant ionic species. To determine which components of the medium and metabolic products significantly influence pH, only species with acid dissociation constants $pK_a < 9$ were considered, as these exhibit appreciable dissociation under physiological conditions.

The following algebraic charge balance equation is solved at each time point to obtain the proton concentration $[H^+]$ (mol/L), which is the only unknown. More details in the Supplementary Material Sections B and C.

$$\begin{aligned}[H^+] = {} & [OH^-] + [KHPO_4^-] + 3[C_6H_5O_7^{3-}] \\ & + 2[C_6H_6O_7^{2-}] + [C_6H_7O_7^-] + [HCO_3^-] - pH_{\text{alk}}\end{aligned} \tag{6}$$

Once $[H^+]$ is determined, the pH is calculated as:

$$\text{pH} = -\log_{10}[H^+] \tag{7}$$

The specific growth rate μ is defined as a function of time and is governed by multiple biological and environmental factors. The expression integrates five multiplicative terms that account for lag phase, substrate availability, oxygen limitation, self-inhibition due to biomass accumulation, and pH-dependent inhibition.

$\mu_{\max}$ is the maximum specific growth rate (1/h), t is time (h), and t_{lag} is the characteristic lag time (h), representing the initial adaptation period. The terms C, N and O are each modulated by their half-saturation constants K_C, K_N and K_O (g/L), respectively. $X_{\max}$ is the maximum biomass capacity (g/L), which models self-inhibition through logistic kinetics. The effect of extracellular pH on microbial growth is represented by the dimensionless inhibition factor $I(\text{pH})$, which penalizes deviations from the physiologically optimal pH range.

$$\mu = \mu_{\max}\left(1 - e^{-\frac{t}{t_{\text{lag}}}}\right) \cdot \frac{C}{C + K_C} \cdot \frac{N}{N + K_N} \cdot \frac{O}{O + K_O} \cdot \left(1 - \frac{X}{X_{\max}}\right) \cdot I(\text{pH}) \tag{8}$$

This last term reduces the specific growth rate under both acidic and alkaline stress, and is defined by three key parameters: the lower and upper pH bounds, pH_{LL} and pH_{UL} (dimensionless), which establish the viable range for growth, and an inhibition shape constant I_{val} (dimensionless), which determines the sensitivity of the system to pH deviations. The expression used is the following:

$$I(\text{pH}) = e^{\left(I_{\text{val}} \cdot \frac{\text{pH} - \text{pH}_{\text{UL}}}{\text{pH}_{\text{UL}} - \text{pH}_{\text{LL}}}\right)^2} \tag{9}$$

C. Software Implementation

To simulate, calibrate and analyze the proposed dynamic model, a Python package—`DAE-Model-Calibration-and-Analysis`—was developed using CasADi, which provides a symbolic framework for defining and solving index-1 DAEs algebraic constraints. Numerical integration is handled by the IDAS solver from the SUNDIALS suite, ensuring robust treatment of the pH charge-balance equations. By supplying the model's DAE expressions and system metadata, the package automatically:

1) Constructs the symbolic DAE system.
2) Performs numerical simulation and compares outputs to validation data.
3) Computes local sensitivities via finite differences.
4) Assembles and inverts the Fisher Information Matrix to derive parameter t-values.
5) Run Particle Swarm Optimization within user-defined bounds for parameter calibration.
6) Re-simulates and re-validates the optimized model and performs further parameter analysis for iterative refinement.

The package can be applied to any DAE formulation by adjusting the equation definitions and parameter specifications. Source code and documentation are available on GitHub: DAE-Model-Calibration-and-Analysis Package.

1) Statistical Validation and Parameter Analysis of the Model: Model validation was conducted by comparing simulations to experimental validation data using Root Mean Square Error (RMSE), Normalized Mean Squared Relative Error (NMSRE), Mean Absolute Percentage Error (MAPE), Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC) metrics. Local sensitivity analysis perturbed each parameter by $\pm 10\%$ around its nominal value and computed time-averaged normalized finite differences to identify the most influential parameters. Practical identifiability was then assessed by assembling the Fisher Information Matrix from sensitivity matrices and error covariances, inverting it to obtain parameter variances, and calculating t-values (parameter estimate divided by its standard error). Parameters with high sensitivity, low correlation and $|t| > 2$ were selected for calibration. Normality, correlation, and comparison to experimental data plots are also generated for a visual analysis.

2) Parameter Estimation: Calibration of the refined DAE model was performed using Particle Swarm Optimization (PSO) to minimize the sum of squared errors between simulated and experimental trajectories of biomass X, glycerol C, ammonium N and pH. Guided by the prior sensitivity and FIM analyses, four parameters—$X_{\max}$, $Y_{X/N}$, pH_{UL} and $\mu_{\max}$—were chosen as decision variables within bounds found in Supplementary Material Section H.

The PSO settings were: 50 particles, 200 iterations, cognitive coefficient $c_1 = 2.0$, social coefficient $c_2 = 1.5$, and inertia weight $\omega = 0.5$. Ten independent runs were conducted to ensure convergence stability. After each run, the optimized parameter set was applied to the full DAE system:

For each perturbed parameter set, the model was simulated and relative sensitivities were computed at every time point. The sensitivities S_{ij} for parameter j and output i were defined as the time-averaged normalized finite difference, as described in [8]:

$$S_{ij} = \frac{1}{T}\int_0^T \left|\frac{y_i^+(t) - y_i^-(t)}{2\delta \cdot y_i(t)}\right| dt \tag{10}$$

Where $y_i^+(t)$ and $y_i^-(t)$ are the model outputs obtained with the increased and decreased values of parameter j, respectively, $\delta = 0.10$ is the magnitude of the perturbation, and T is the simulation time horizon. In practice, the integral in (10) was approximated using the discrete average across all simulation time points.

For the FIM analysis, simulations are run utilizing the initial conditions given by experimental data for parameter estimation. As described in [8], the FIM is defined as:

$$\text{FIM} = \sum_{l=1}^{N} \mathbf{G}(t_l)^T \cdot \mathbf{Q_l}^{-1} \cdot \mathbf{G}(t_l) \tag{11}$$

Each optimized set was validated by recomputing RMSE, NMSRE, MAPE, AIC and BIC, and updating the FIM for fresh t-value estimates.

Note that pKa values, the fixed concentrations of phosphate and citric acid, and the ionic contribution term pH_{alk} were treated as constant parameters, as their values are determined by well-established chemical equilibria or experimental preparation protocols, and do not vary under the simulated conditions. Furthermore, preliminary tests confirmed that their influence on the model outputs was negligible within physiologically relevant ranges.

3) Comparative Simulations: To assess the *M. smegmatis* model's ability to capture relevant biological dynamics, particularly those related to oxygen availability, two distinct simulation conditions were implemented. Firstly, an oxygen-saturated environment, consistent with the experimental setup used in the original model, was employed. For this simulation, the parameter values estimated in that study were retained. Secondly, a hypoxic environment was designed to mimic physiological contexts such as granuloma formation. In this case, the volumetric oxygen transfer coefficient k_La was reduced to represent poor gas-liquid transfer, and a low initial dissolved oxygen concentration was set to reflect a pre-existing hypoxic state.

A comparative analysis was then performed between three model configurations: (i) the base model prior to oxygen integration, (ii) the oxygen-enriched scenario, and (iii) the hypoxia simulation. This comparison enabled the evaluation of the impact of oxygen availability on microbial growth, substrate consumption, pH evolution, and overall system dynamics.

D. Initial parameters and conditions

Initial parameter values and state concentrations used in the model correspond to those reported by [7], ensuring consistency with the original experimental setup. Initial conditions were defined separately for the fitting and validation datasets. A summary of the model parameters and constants, as well as the initial conditions for both datasets, is provided in Supplementary Material Section E.

III. Results & Discussion

A. Oxygen-Limited and Hypoxic Models

Extending the original DAE framework to include dissolved-oxygen dynamics produced marked changes in system behavior. Under oxygen-limited conditions (nominal k_La, high initial O_2), the culture required approximately 40 h to stabilize, during which dissolved oxygen fell to 0.0073 mg/L. This oxygen depletion slowed glycerol and ammonium uptake, yielded more gradual CO_2 production and delayed the pH drop, while the peak specific growth rate μ declined relative to the oxygen-unlimited case.

Under hypoxic conditions ($k_La = 5$ h^{-1}, initial $O_2 = 0.0001$ g/L), these trends were intensified: stabilization extended to 55 h, final biomass remained near 1.442 g/L, but substrate consumption and CO_2 generation slowed further. The maximum μ fell to 0.060 h^{-1}, a 72% reduction compared to the original oxygen-unlimited model and 28% lower than the oxygen-limited scenario.

These results demonstrate that *M. smegmatis* adapts to decreasing oxygen availability by prolonging growth phases and reducing metabolic rates, consistent with its facultative aerobic physiology and observations of hypoxic adaptation in *M. tuberculosis* granulomas. Time-course comparisons of biomass $X(t)$, glycerol $C(t)$, ammonium $N(t)$, $CO_2(t)$ and $\text{pH}(t)$ for the base-case, oxygen-limited and hypoxic models are shown in Figure S1.

B. Original Model Validation and Parameter Analysis

The extended model (including oxygen dynamics), from now on referred as the "Original model", was validated against experimental data (Figure S2) and compared to the original formulations of Apiyo [7] and De Witt [10]. Validation metrics (Tables I and II) show that the De Witt model outperforms Apiyo's across all variables, while the oxygen-augmented model further improves biomass and glycerol fits. Specifically, the oxygen-inclusive model achieves lower NRMSE for glycerol and biomass, and its AIC (–110.18) is slightly more favorable than De Witt's (–104.20).

TABLE I
NRMSE per variable for Model Fitting Under Different Parameter Sets

Variable	Apiyo [7]	De Witt [10]	Original	Calibrated
Biomass	35.686%	21.08 %	17.897 %	8.257 %
Glycerol	23.712 %	8 %	6.275 %	3.370 %
Ammonia	27.629%	6.15 %	6.473 %	1.368 %
pH	13.399 %	1.43	1.448 %	0.586 %

TABLE II
Model Validation Metrics Under Different Parameter Sets

Variable	De Witt [10]	Original	Calibrated
AIC	-104.202	-110.177	-158.373
NRMSE	9.167	8.023	3.395

A local sensitivity analysis (Supplementary Figure S4) quantified each parameter's influence by perturbing them ±10% around their nominal values and computing time-averaged normalized finite differences. Parameters pH_{UL} and $X_{\max}$ exhibited the highest sensitivities across all outputs and were selected for calibration. Secondary relevant parameters included $Y_{X/C}$ (exclusively for glycerol), $Y_{X/N}$ (exclusively for ammonia) and Y_{X/CO_2} (exclusively for pH). In contrast, parameters such as k_C, k_N, k_O, k_d, Y_{X/O_2} and k_La showed negligible impact. The combined roles of pH_{UL}, pH_{LL} and I_{val} underscore the importance of pH homeostasis

for a neutrophilic organism, while $X_{\max}$ defines the carrying capacity and $\mu_{\max}$ dictates exponential growth dynamics.

Upon seeing the visual comparison of the original model with the experimental validation data (Figure S2), there is a high discrepancy between data and simulations for ammonia dynamics, and also for the maximum biomass obtained, indicating areas for further model refinement and calibration.

C. Parameter Estimation

Calibration of the refined DAE model was carried out in two sequential stages using PSO to minimize the sum of squared residuals between simulated and experimental time courses of biomass $X(t)$, glycerol $C(t)$, ammonium $N(t)$ and pH (variables for which experimental data was available).

In the first stage, the carrying capacity $X_{\max}$ and the ammonia yield coefficient $Y_{X/N}$ were treated as decision variables. The bounds used were $X_{\max} \in [1.1, 1.3]$ and $Y_{X/N} \in [5, 8]$. Ten independent PSO runs were performed to assess convergence stability. The best solutions converged to $X_{\max} = 1.1829 g/L$ and $Y_{X/N} = 8.0$ g X/g N. After each run, updated parameters were substituted into the full DAE system and validation metrics (RMSE, NMSRE, MAPE, AIC, BIC) were recomputed to confirm the improvement in ammonia and biomass fits. Details of the process are in Supplementary Material Section H.1.

In the second stage, with $X_{\max}$ and $Y_{X/N}$ fixed at their optimized values, the upper pH bound $\mathrm{pH_{UL}}$ and the maximum specific growth rate $\mu_{\max}$ were calibrated. Search bounds were set to $\mathrm{pH_{UL}} \in [6.5, 7.5]$ and $\mu_{\max} \in [0.1, 0.5]\,\mathrm{h}^{-1}$. Using identical PSO settings and ten independent runs, calibration converged to $\mathrm{pH_{UL}} = 6.9$ and $\mu_{\max} = 0.30\ \mathrm{h}^{-1}$, lowering the AIC to –158.4, a 1.5-fold improvement over the first stage. Each optimized pair was re-simulated in the full model (including oxygen and pH dynamics) to recompute all validation metrics and to assemble the Fisher Information Matrix for updated t-value estimation.

All four parameters achieved $|t| > 2$, confirming their statistical significance (Table III). Notably, NRMSE values dropped to 8.26% for biomass, 3.37% for glycerol, 1.37% for ammonia, and 0.59% for pH, although residual parameter correlations suggest further refinement—potentially via sequential or multiobjective optimization—may be beneficial.

TABLE III
T VALUES FOR PARAMETERS IN CALIBRATIONS

Parameters	t-values
$X_{\max}$	11899.58
$Y_{X/N}$	1154.42
$\mathrm{pH_{UL}}$	18291.44
$\mu_{\max}$	2811.88

These improvements demonstrate that the calibrated parameter set enhances the model's ability to reproduce experimental dynamics. The consistent error reductions across biomass, substrate, and pH outputs confirm the efficacy of the sensitivity-guided calibration approach.

IV. CONCLUSIONS

In this study, the differential–algebraic equation (DAE) model of *Mycobacterium smegmatis* batch growth was extended to include an explicit mass balance for dissolved oxygen, enabling accurate simulation of oxygen-limited and hypoxic environments. A CasADi-based Python package, `DAE-Model-Calibration-and-Analysis`, was developed to automate DAE formulation, numerical simulation, sensitivity analysis, Fisher Information Matrix computation, and Particle Swarm Optimization–based parameter calibration.

Sensitivity and identifiability analyses guided the calibration of four key parameters—$X_{\max}$, $Y_{X/N}$, $\mathrm{pH_{UL}}$ and $\mu_{\max}$. Optimal values ($X_{\max} = 1.1829$, $Y_{X/N} = 8.0$, $\mathrm{pH_{UL}} = 6.9$, $\mu_{\max} = 0.30$) reduced individual NRMSEs to single-digit values (biomass: 17.9% to 8.3%; glycerol: 6.3% to 3.4%; ammonia: 6.5% to 1.4%; pH: 1.5% to 0.6%) and overall model NRMSE from 8.0% to 3.4%, while lowering AIC from –110.2 to –158.4. All calibrated parameters achieved $|t| > 2$, confirming their statistical significance.

The refined model reproduces hallmark behaviors such as delayed biomass accumulation, slowed substrate uptake, and gradual pH shifts under both oxygen-replete and hypoxic conditions, demonstrating its mechanistic fidelity and numerical stability.

Future work will focus on integrating additional stressors (e.g., nutrient deprivation, acid challenge), exploring alternative or sequential calibration strategies to reduce parameter correlations. Additionally, the package's optimization robustness can be enhanced, and its functionality can be extended to support multiobjective workflows. The developed model can be applied to simulate granuloma-like micro-environments to explain and design targeted *in vitro* and *in vivo* studies in tuberculosis research.

DATA AVAILABILITY

The experimental datasets used for model calibration and validation were supplied by the research group in De Witt et al. [10], based on the original measurements collected by Dr. Apiyo [7].

SUPPLEMENTARY MATERIAL

Supplementary Material is available at: https://github.com/Laefivy/M.smegmatis.

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