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Identification of microbial batch growth dynamics in Chi.Bio mini-bioreactors

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Resumen

Este trabajo presenta la identificación de la dinámica de crecimiento microbiano en experimentos en lote realizados en mini-biorreactores Chi.Bio. El objetivo es estimar los parámetros de un modelo no lineal sencillo que describe el crecimiento de una población microbiana que consume glucosa como sustrato limitante. Para ello, las medidas de densidad óptica se convierten primero en concentración de biomasa mediante una calibración basada en el número equivalente de partículas y la masa seca celular. Posteriormente, se ajusta un modelo de crecimiento tipo Monod, que incluye una concentración máxima efectiva de biomasa, utilizando experimentos realizados con diferentes concentraciones iniciales de glucosa. Los parámetros del modelo se estiman minimizando el error entre las trayectorias experimentales de biomasa y las respuestas simuladas del modelo. Los resultados muestran que el modelo ajustado reproduce adecuadamente las curvas de crecimiento, alcanzando un valor de $R^2 = 0.981$ y un RMSE global de 0.045 g/L. Estos resultados indican que la metodología propuesta permite obtener un modelo dinámico compacto del crecimiento microbiano en lote a partir de medidas obtenidas en la plataforma Chi.Bio.

Palabras clave: System identification, Parameter estimation, Bioprocesses, Biological systems, Nonlinear systems

Identificación de la dinámica de crecimiento microbiano en lote en mini-biorreactores Chi.Bio

Abstract

This work presents the identification of microbial batch growth dynamics using experiments performed in Chi.Bio mini-bioreactors. The objective is to estimate the parameters of a simple nonlinear model describing the growth of one microbial population consuming glucose as the limiting substrate. Optical density measurements are first converted into biomass concentration using a calibration based on equivalent particle counts and dry cell mass. Then, a Monod-type growth model, including an effective maximum biomass concentration, is fitted to batch experiments performed at different initial glucose concentrations. The model parameters are estimated by minimizing the error between the measured biomass trajectories and the simulated model response. The fitted model reproduces the experimental growth curves with good accuracy, achieving an R^2 value of 0.981 and a global RMSE of 0.045 g/L. The results show that the proposed approach can be used to obtain a compact dynamic model of microbial batch growth from Chi.Bio measurements.

Keywords: System identification, Parameter estimation, Bioprocesses, Biological systems, Nonlinear systems

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1. Introduction

Bioreactors play an important role in biotechnology, microbiology, synthetic biology, and biochemical engineering, because they provide a controlled environment for the cultivation of microbial populations and for the study of biochemical reactions (Keasling et al., 2021; Liao et al., 2018; Palladino et al., 2024). Depending on the operation mode, bioreactors can be used in batch, fed-batch, or continuous culture. In batch mode, a fixed amount of nutrients is provided at the beginning of the experiment and is progressively consumed by the microorganisms. This operation mode is simple and widely used to characterize microbial growth dynamics.

In recent years, small-scale and open-source bioreactors have become increasingly relevant for laboratory research. These platforms allow experiments to be carried out under controlled and reproducible conditions, while reducing cost and experimental volume. In particular, they are useful in synthetic biology, where biological systems are often designed and improved using Design-Build-Test-Learn iterative strategies (Hemmerich et al., 2018; Steel et al., 2020; Denton et al., 2024; Beal et al., 2022).

The Chi.Bio platform is an open-source mini-bioreactor designed for automated microbial culture experiments (Steel et al., 2020). It allows online monitoring and control of key culture variables such as temperature, stirring, optical density, and fluorescence. Due to its modular design, Chi.Bio can be used to perform several experiments in parallel and to obtain time-series data from microbial cultures. Figure 1 shows the Chi.Bio mini-bioreactor used in this work.

Optical density measurements are commonly used to monitor bacterial growth, since they provide a simple and non-invasive indication of the amount of biomass in the culture. However, optical density is an indirect measurement and is usually expressed in arbitrary units. Therefore, it is not directly comparable between different instruments or experimental setups. To improve the interpretation of these measurements, optical density can be converted into standardized units based on equivalent particle concentration (Boada et al., 2019; Beal et al., 2020). This calibration step makes it possible to relate optical measurements to biomass concentration, which is required for mathematical modeling.

Mathematical models are useful tools to describe, predict, and analyze microbial growth. In particular, substrate-dependent growth models, such as the Monod model, are commonly used to represent the relation between nutrient availability and microbial growth rate (Monod, 1949). These models can be combined with experimental data to estimate relevant biological parameters, such as the maximum growth rate, the half-saturation constant, the biomass yield, and the maximum attainable biomass concentration.

In this work, a nonlinear batch growth model is identified from experiments performed with *E. coli* 10G cultures in Chi.Bio mini-bioreactors. The experiments were carried out using different initial glucose concentrations, with glucose acting as the limiting substrate. Optical density measurements were converted into biomass concentration using a standardized calibration procedure. Then, the resulting biomass trajectories were used to estimate the parameters of a Monod-type batch growth model.

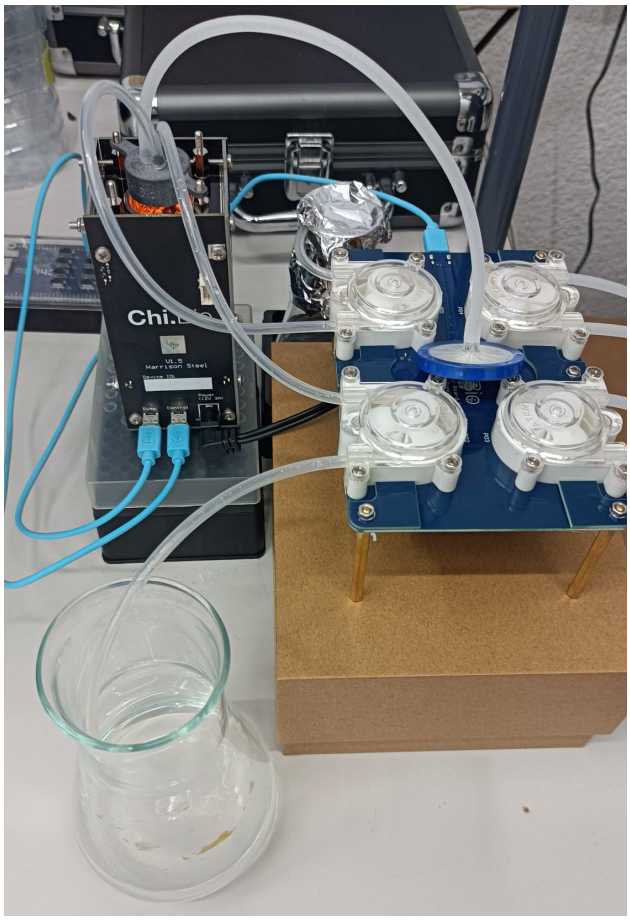


Figure 1: Chi.Bio open-source mini-bioreactor platform used for online monitoring of microbial batch growth experiments. The system allows controlled cultivation and acquisition of optical density measurements during the experiment.

The objective is to obtain a compact dynamic model able to reproduce the observed microbial growth curves under different substrate conditions.

The rest of the paper is organized as follows. Section 2 presents the mathematical model used to describe batch microbial growth. Section 3 describes the conversion from optical density to biomass concentration, the experimental dataset, and the parameter identification procedure. Section 4 presents the identification results and discusses the quality of the model fit. Finally, Section 5 summarizes the main conclusions and future work.

2. The model

We consider one microbial population growing in a batch reactor by consuming one limiting substrate. The dynamics are described by

$$\begin{cases} \dot{x} = \mu(x, s)x \\ \dot{s} = -\dfrac{1}{Y}\mu(x, s)x \end{cases} \tag{1}$$

where $x \in \mathbb{R}_{\geq 0}$ denotes the concentration of the biomass, and $s \in \mathbb{R}_{\geq 0}$ denotes the concentration of the substrate. In the experiments considered in this work, the limiting substrate is glucose. Moreover, Y is the yield coefficient, defined as the

ratio between the biomass produced and the substrate consumed. As in (Fiore et al., 2021), the culture is assumed to be well mixed, and mortality and attachment of the bacteria are neglected. Under these assumptions, $\mu\colon \mathbb{R}_+^2 \to \mathbb{R}_+$ is a nonlinear function representing how the biomass grows inside the reactor. This rate is assumed to depend both on substrate availability and on the current biomass concentration according to

$$\mu(x, s) = \mu_s(s)\Big(1 - \frac{x}{x_{\max}}\Big), \tag{2}$$

where $x_{\max} > 0$ is the effective maximum biomass concentration. The substrate-dependent term $\mu_s(s)$ is modeled by the Monod law (Monod, 1949),

$$\mu_s(s) = \mu^* \frac{s}{k + s}, \tag{3}$$

where μ^* is the maximum substrate-dependent growth rate and k is the half-saturation constant. Combining (2) and (3), the effective specific growth rate is therefore given by

$$\mu(x, s) = \mu^* \frac{s}{k + s}\Big(1 - \frac{x}{x_{\max}}\Big). \tag{4}$$

3. Estimation and identification

The parameters of (1) are estimated from several experiments in a Chi.Bio architecture performed at different initial substrate concentration.

3.1. Estimation of the biomass from OD

To estimate the biomass concentration $x(t)$, OD measurements are first converted into an estimated number of particles, which is then converted into biomass concentration. The conversion from OD to particle number is based on the microsphere calibration protocol proposed in (Beal et al., 2020), where one particle is taken as equivalent to one *E. coli* cell, and adapted specifically to Chi.Bio as in (Díaz-Iza et al., 2025). In this protocol, serial dilutions of silica microspheres with known particle number are used to relate optical density measurements to equivalent particle counts. This equivalence is appropriate for the present experiments because the biological system under study is based on *E. coli* 10G cultures. Namely, the equivalent number of particles is computed according to

$$N(t) = 10^{p_0+2}(0.55\,\mathrm{OD}_{600}(t))^{p_1}, \tag{5}$$

where p_0 and p_1 are empirical calibration parameters reported in (Boada et al., 2019), namely $p_0 = 8.062$ and $p_1 = 1.185$. The biomass concentration is then estimated as

$$x(t) = \frac{m_{\mathrm{cell}}}{V} N(t), \tag{6}$$

where m_{cell} is the estimated dry mass of a single *E. coli* cell, and V is the culture volume of the Chi.Bio. The dry mass per cell is assumed to remain approximately constant during the experiment. Therefore, a representative value of dry mass measurement for *E. coli* is used, namely $m_{\mathrm{cell}} = 2.8 \cdot 10^{-13}$ g/cell, reported for *E. coli* B/r in glucose medium in (Neidhardt, 1996), and the volume is set to $V = 20$ mL as is the nominal volume of the Chi.Bio.

Table 1: Batch experiments used for parameter identification.

Condition	Glucose percentage	s_0 [g/L]
1	0.555%	1
2	1.11%	2
3	1.665%	3
4	2.22%	4
5	8.88%	16

3.2. Experimental dataset

The identification was performed using batch experiments with *E. coli* 10G cultures carried out in the Chi.Bio platform at different initial substrate concentrations. In all experiments, the limiting substrate was glucose, so that the variable s in the model corresponds to the glucose concentration. The initial glucose concentrations were selected to cover both low and high substrate growth conditions.

The dataset consists of five biomass trajectories, obtained from calibrated OD measurements as described in Section 3.1. The initial substrate concentrations used for identification are reported in Table 1.

3.3. Identification of the parameters

Since both equations in (1) are coupled only through the product $\mu(x, s)\,x$, dividing the substrate equation by the biomass equation yields $\dot{s} = -\dot{x}/Y$, which integrates to

$$s(t) = s_0 - \frac{x(t) - x_0}{Y}, \tag{7}$$

where s_0 and x_0 denote the initial substrate and biomass concentrations, respectively. Substituting (7) into (2), the batch model can be reduced to the scalar ODE

$$\dot{x} = \mu^* \frac{s_0 - (x - x_0)/Y}{k + s_0 - (x - x_0)/Y}\Big(1 - \frac{x}{x_{\max}}\Big)x. \tag{8}$$

The parameter vector to be estimated is therefore

$$\theta = (\mu^*, k, Y, x_{\max}) \in \mathbb{R}^4_{>0}. \tag{9}$$

Let M denote the number of batch experiments used for the identification. For each experiment $j = 1, \ldots, M$, let

$$\Big\{(t_i^{(j)}, x_i^{(j)})\Big\}_{i=1}^{N_j} \tag{10}$$

be the measured biomass trajectory estimated as in Sec. 3.1, where N_j is the number of samples in experiment j. Each experiment is characterized by its own initial biomass concentration $x_0^{(j)}$ and initial substrate concentration $s_0^{(j)}$, corresponding in our case to the initial glucose concentration. For a given parameter vector θ, we denote by

$$x^{(j)}(t; \theta) \tag{11}$$

the solution of the scalar batch model (8) initialized with $x_0^{(j)}$ and $s_0^{(j)}$. The mean squared error associated with experiment j is defined as

$$\mathrm{MSE}_j(\theta) = \frac{1}{N_j} \sum_{i=1}^{N_j} \Big(x_i^{(j)} - x^{(j)}(t_i^{(j)}; \theta)\Big)^2. \tag{12}$$

The normalization by N_j assigns the same weight to each experiment independently of the number of sampled time points. The identification problem is then formulated as

$$\hat{\theta} = \arg\min_{\theta \in \Theta} \sum_{j=1}^{M} \mathrm{MSE}_j(\theta), \tag{13}$$

where Θ is the feasible set defined as

$$\Theta = \{\theta \in \mathbb{R}^4 : \mu^* > 0,\ k > 0,\ Y > 0, x_{\max} > 0\}. \tag{14}$$

The optimization problem (13) was solved in MATLAB using `lsqcurvefit` from the Optimization Toolbox. The initial parameter vector was

$$\theta_0 = (5, 1, 1, 1), \tag{15}$$

with units consistent with (9). The optimization was constrained using broad physically admissible bounds, selected to guarantee positivity of all parameters while remaining sufficiently wide with respect to the expected biological ranges:

$$\theta_{\min} = (10^{-5}, 10^{-5}, 10^{-5}, 0.05), \quad \theta_{\max} = (10, 20, 5, 5). \tag{16}$$

Local 95% confidence intervals were computed from the Jacobian of the weighted nonlinear least-squares problem at the optimum. The fit quality is evaluated using the coefficient of determination R^2 and the global root mean squared error RMSE, defined as

$$\mathrm{RMSE} = \sqrt{\frac{1}{M} \sum_{j=1}^{M} \mathrm{MSE}_j(\hat{\theta})}, \tag{17}$$

consistently with (13).

4. Results

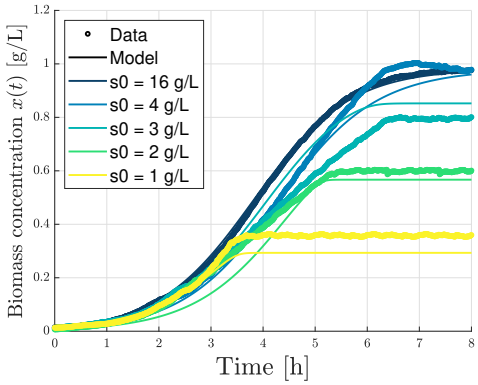
The parameter identification was performed using the five batch experiments reported in Table 1. The estimated parameters are shown in Table 2. The fitted model achieved a coefficient of determination $R^2 = 0.981$ and RMSE = 0.045 g/L.

The measured biomass trajectories and the corresponding fitted model trajectories are reported in Figure 2(a). The fitted model reproduces the growth curves across the different initial glucose concentrations. In particular, the trajectories with lower initial substrate concentrations reach lower biomass levels, whereas the high-substrate experiments approach a common upper biomass level close to the estimated value of $x_{\max}$. Moreover, Figure 2(b) reports the substrate trajectories reconstructed from the mass balance (7). According to the identified model, the low-glucose experiments are compatible with substrate depletion, while the high-glucose experiments approach the effective biomass limit $x_{\max}$ with residual substrate.

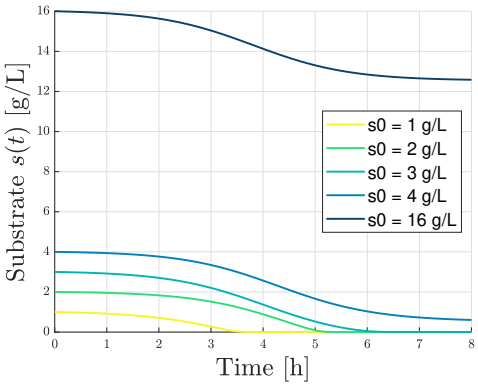
The identified substrate-dependent growth function is shown in Figure 3. The estimated value $k = 0.060$ g/L indicates that the Monod term reaches near-saturation already at relatively low substrate concentrations. Therefore, for the largest initial substrate concentrations used in the experiments, the substrate-dependent growth rate is close to its maximum value μ^* during the early phase of the batch.

Table 2: Estimated parameters of (1) with local 95% confidence intervals.

Parameter	Estimate [95% CI]	Unit
μ^*	1.099 [1.094, 1.104]	h^{-1}
k	0.060 [0.051, 0.069]	g/L
Y	0.280 [0.279, 0.281]	–
$x_{\max}$	0.981 [0.978, 0.984]	g/L



(a)



(b)

Figure 2: Batch model reconstruction obtained with the identified parameters as in Table 2. Panel (a) shows the measured biomass and the corresponding fitted model trajectories, at different initial substrate concentrations as in Table 1. Panel (b) shows the substrate trajectories reconstructed from the mass balance relation (7).

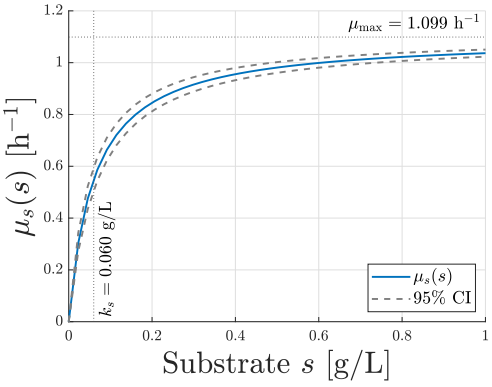


Figure 3: Identified Monod growth function $\mu_s(s)$ as in (3), using parameters as in Table 2.

5. Conclusions

This work presented a methodology to identify microbial batch growth dynamics from *E. coli* 10G experiments performed in Chi.Bio mini-bioreactors. Optical density measurements were converted into biomass concentration through an OD-to-particle calibration and a dry-mass-per-cell conversion. The resulting trajectories were used to estimate the parameters of a Monod-type batch growth model with an effective maximum biomass concentration.

The identified model captures the main features of the experimental trajectories across the tested glucose concentrations. In particular, the fitted dynamics distinguish between low-glucose conditions, where growth is compatible with the total substrate consumption, and high-glucose conditions, where the biomass approaches an upper limit. However, the method assumes an equivalence between calibration particles and bacterial cells, uses a representative value for the dry mass of an *E. coli* cell, and assumes that this value remains approximately constant during the experiment. These assumptions may affect the absolute values of the estimated parameters.

Overall, the results show that Chi.Bio mini-bioreactors, when combined with standardized optical density calibration and model-based parameter identification, can provide valuable information about microbial growth dynamics. This supports the use of small-scale bioreactor platforms not only for experimental monitoring, but also for the construction of quantitative models that can assist the analysis, prediction, and future control of biological cultures.

Future work will focus on validating the reconstructed substrate dynamics through independent measurements of residual glucose. In addition, direct biomass quantification could be used to further assess the accuracy of the calibration procedure. These additional measurements would help distinguish between biological limitations of the culture and possible limitations of the optical density reader. Finally, the proposed identification approach could be extended to more complex experimental conditions, including fed-batch or continuous operation, additional substrates, and engineered microbial strains with inducible genetic circuits.

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