Supplementary information

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PhysioFit: a software to quantify cell growth parameters and extracellular fluxes

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This document describes the procedure used to evaluate PhysioFit and the validation results.

1. Evaluation procedure

To validate PhysioFit, we implemented three published flux models and calculated fluxes from the accompanying datasets, as described in the original publications. We compared the growth rate and extracellular fluxes obtained with PhysioFit to the published values.

We chose to implement the following models:

- Model 1 : a steady-state model for batch cultivation, where extracellular fluxes and growth rate are constant during the exponential growth phase (Berges, et al., 2021)
- Model 2 : a steady-state model for batch cultivation, where extracellular fluxes and growth rate are constant during the exponential growth phase, with additional (abiotic) degradation of a substrate that follows a first-order kinetic (Peiro, et al., 2019)
- Model 3 : a dynamic model for batch cultivation, where extracellular fluxes and growth rate change during the cultivation following a Monod kinetic (Zentou, et al., 2019)

These models, which are detailed in the next sections and in the original publications, cover typical situations that are encountered in metabolic studies. The three datasets are available at https://github.com/MetaSys-LISBP/PhysioFit in folder ./validation/models/.

As detailed below, results obtained using PhysioFit were in good agreement with published results, thereby validating the software and the models implemented.

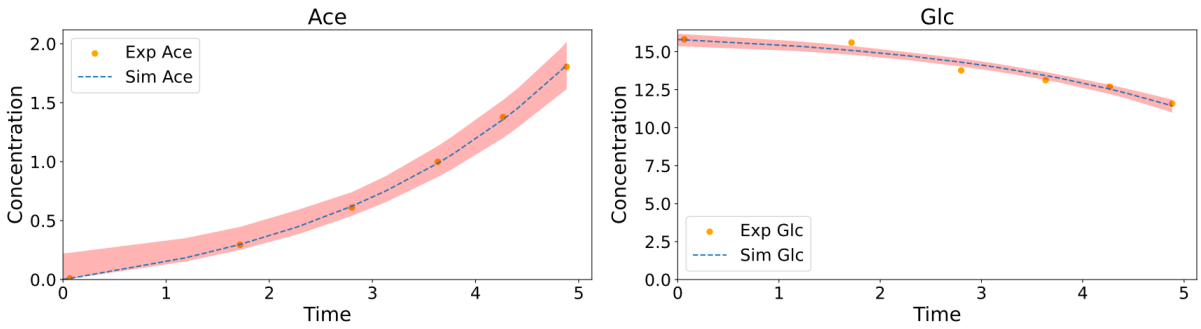
2. Steady-state model

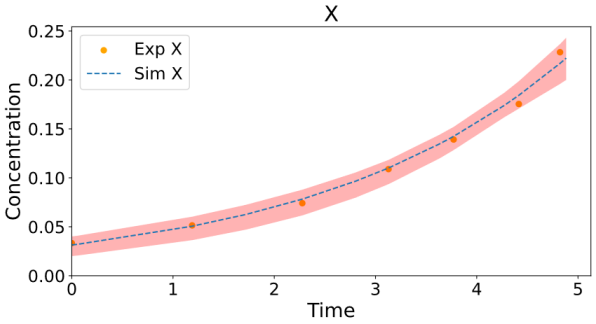
We implemented a model to calculate steady-state fluxes and growth rate in exponential growth phase during batch cultivations of microorganisms. We applied this model to calculate glucose uptake, acetate production and growth rate of *Escherichia coli* BW25113 Δ*ymjA* grown on glucose in M9 medium, as detailed in (Berges, et al., 2021). The time-course concentrations of glucose (Glc), acetate (Ace) and biomass (X) were taken from the publication. Fluxes were calculated using the following model:

$$X(t) = X_0 \cdot e^{\mu \cdot t}$$

$$M(t) = q_M \cdot \frac{X_0}{\mu} \cdot (e^{\mu \cdot t} - 1) + M_0$$

Simulation results for the best fit (see below) are in good agreement with the experimental data.





The initial concentration of each species (*X_0*, *Glc_0*, *Ace_0*) and the fluxes (growth rate; glucose uptake rate, *Glc_q*; and acetate production rate, *Ace_q*) are provided below with their associated confidence intervals.

	PhysioFit v3.1.0						Berges et al., 2020		relative difference
parameter	**optimal**	**mean**	**sd**	**median**	**CI_2.5**	**CI_97.5**	**val**	**sd**	
X_0	0.031	0.031	0.006	0.031	0.020	0.040	0.031	0.005	$-6.4 \cdot 10^{-6}$
growth_rate	0.401	0.407	0.045	0.400	0.337	0.495	0.401	0.041	$3.8 \cdot 10^{-6}$
Glc_q	-9.180	-9.367	0.920	-9.271	-11.576	-7.717	-9.180	0.956	$1.7 \cdot 10^{-6}$
Glc_0	15.801	15.798	0.216	15.790	15.363	16.176	15.801	0.203	$-3.2 \cdot 10^{-7}$
Ace_q	3.820	3.781	0.390	3.709	3.021	4.549	3.820	0.376	$2.9 \cdot 10^{-6}$
Ace_0	0.002	0.036	0.062	0.000	0.000	0.222	0.002	0.059	$-4.9 \cdot 10^{-5}$

The published and calculated fluxes are in excellent agreement, with relative differences below 10^{-4}.

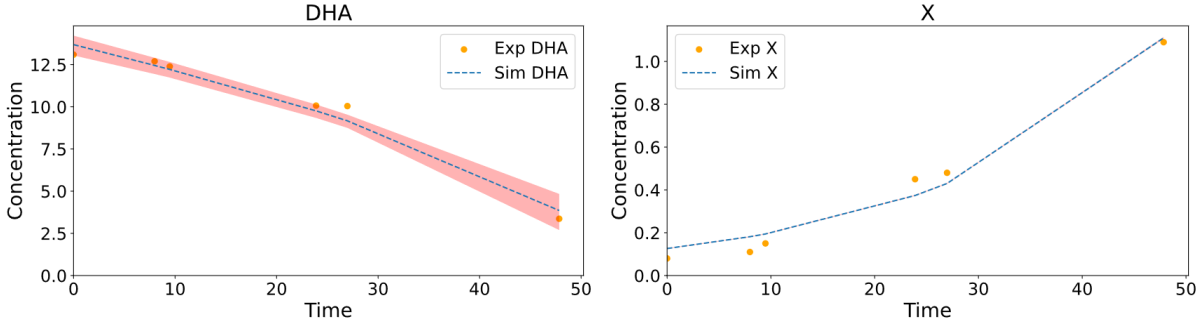
3. Steady-state model with metabolite degradation

We implemented the same model with additional (non-enzymatic) degradation of carbon sources such as DHA or glutamine. The experimental data and the corresponding model were taken from (Peiro, et al., 2019). As detailed in this publication, the dynamics of biomass (X) and metabolite concentrations (DHA) are represented with the following equations.

$$X(t) = X_0 \cdot e^{\mu \cdot t}$$

$$M(t) = q_{Mi} \cdot \frac{X_0}{\mu} \cdot (e^{-k \cdot t} - e^{\mu \cdot t}) + Mi_0 \cdot e^{-k \cdot t}$$

The degradation constant for DHA was set to $8.64 \cdot 10^{-3}$, as determined experimentally in (Peiro, et al., 2019). Simulation results for the best fit (see below) are in good agreement with the experimental data.



The initial concentration of each species (*X_0*, *DHA_0*) and the fluxes (growth rate; DHA uptake rate, *DHA_q*) are provided below with their associated confidence intervals.

	PhysioFit v3.1.0						Peiro et al., 2019		relative difference
parameter	**optimal**	**mean**	**sd**	**median**	**CI_2.5**	**CI_97.5**	**val**	**sd**	
X_0	0.126	0.126	0.001	0.126	0.125	0.128	0.126	0.001	$5.2 \cdot 10^{-5}$
growth_rate	0.045	0.045	0.000	0.045	0.045	0.046	0.045	0.000	$-2.3 \cdot 10^{-5}$
DHA_q	-0.284	-0.273	0.030	-0.270	-0.347	-0.227	-0.287	0.028	-0.013
DHA_0	13.687	13.661	0.293	13.652	13.045	14.218	13.853	0.254	-0.012

Here again, all parameters obtained with PhysioFit are consistent with the published values, with relative differences ranging between -0.013 and $5 \cdot 10^{-5}$.

4. Dynamic (Monod) model

We also implemented a dynamic model where fluxes and growth are represented by Monod kinetics, as detailed in (Zentou, et al., 2019). This model was used to estimate the biomass and ethanol yields of the yeast *Saccharomyces cerevisiae* during a batch fermentation of molasses. The following system of ordinary differential equations was implemented to represent the biomass (X), molasses (S_GlcEq) and ethanol (P_ethanol) dynamics:

$$\frac{dS}{dt} = -X \cdot q_S$$

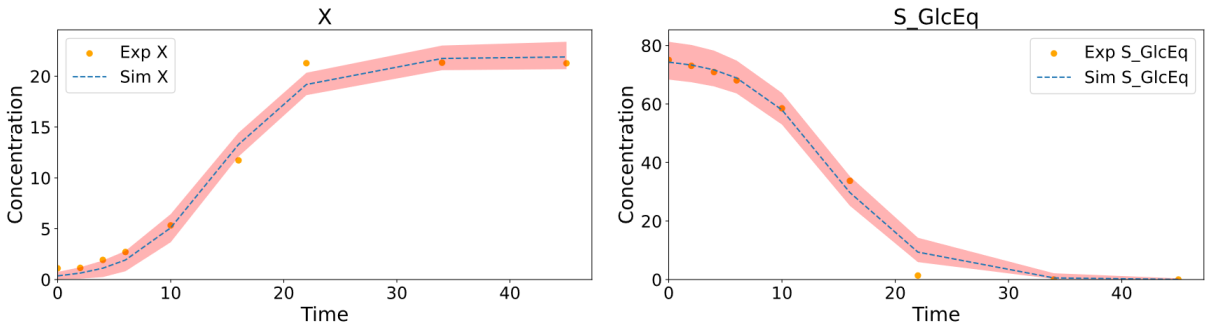
$$\frac{dEtOH}{dt} = q_S \cdot ethanol\_yield$$

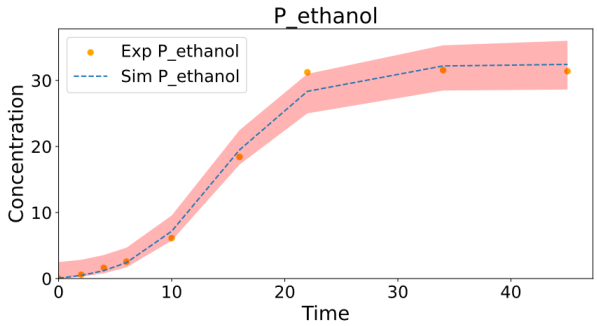
$$\frac{dX}{dt} = q_S \cdot biomass\_yield$$

where the dependence of the molasses uptake rate (q_S) on the molasses concentration (S, expressed in glucose equivalents) is expressed by the following Monod kinetics:

$$q_S = qS_{max} \cdot \frac{S}{K_M + S}$$

Simulation results for the best fit (see below) are in good agreement with the experimental data.





The initial concentration of each species (*X_0*, *S_GlcEq_s_0*, *P_ethanol_p_0*) and the growth parameters (biomass yield, *y_BM*; ethanol yield, *P_ethanol_y_P*; affinity for the substrate, *S_GlcEq_km*; maximal substrate uptake rate, *S_GlcEq_qsmax*) are provided below with their associated confidence intervals.

	PhysioFit v3.1.0						Zentou et al., 2019		relative difference
parameter	**optimal**	**mean**	**sd**	**median**	**CI_2.5**	**CI_97.5**	**val**	**sd**	
X_0	0.359	0.356	0.184	0.337	0.029	0.751	*n.a.*	*n.a.*	
y_BM	0.290	0.293	0.019	0.291	0.261	0.331	0.286	*n.a.*	0.014
S_GlcEq_km	100.000	87.606	28.689	99.921	10.314	100.000	*n.a.*	*n.a.*	
S_GlcEq_qsmax	1.349	1.188	0.379	1.278	0.203	1.651	*n.a.*	*n.a.*	
S_GlcEq_s_0	74.292	74.403	3.528	74.512	68.348	81.255	75	*n.a.*	-0.009
P_ethanol_y_P	0.436	0.431	0.034	0.431	0.365	0.488	0.431	*n.a.*	0.011
P_ethanol_p_0	0.060	0.570	0.870	0.007	0.001	2.490	*n.a.*	*n.a.*	

n.a.: not available in the original publication

Parameters obtained with PhysioFit are consistent with the published values, with relative differences about 1 %.

5. References

Berges, C.*, et al.* Exploring the Glucose Fluxotype of the E. coli y-ome Using High-Resolution Fluxomics. *Metabolites* 2021;11(5).

Peiro, C.*, et al.* Chemical and metabolic controls on dihydroxyacetone metabolism lead to suboptimal growth of *Escherichia coli*. *Appl Environ Microbiol* 2019;85(15):e00768.

Zentou, H.*, et al.* Modelling of molasses fermentation for bioethanol production: a comparative investigation of Monod and Andrews models accuracy assessment. *Biomolecules* 2019;9(8):308.