CITRUSAFE Mathematical Modeling

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1 Overview

This documentation presents the mathematical modeling we employed to determine the theoretical yield rate of the CYP6B1 based on a standard growth curve that Saccharomyces cerevisiae normally follows.

2 Introduction

To estimate the scalability and effectiveness of our project in the real-world application, we set up a standard growth model and an enzyme-substrate interaction model to determine the theoretical CYP6B1 yield rate of our engineered Saccharomyces cerevisiae.

The standard growth model is implemented based on the current standardization of yeast growth curves available online.

The enzyme-substrate interaction model uses Michaelis–Menten kinetics to estimate the rate of interaction between the CYP6B1, CYP6B1 reductase, and the furanocoumarin.

For the rest of the document, we will abbreviate CYP6B1 as "CYP", CYP6B1 reductase as "CYP reductase", and the furanocoumarin as "FC".

3 Yeast Growth Curve Modeling

We used MATLAB for the growth curve modeling. The rationale and the mathematical equations for the growth curve modeling are based on the work that was previously done by the Division of Mathematical Statistics (Department of Mathematical Sciences) at Chalmers University of Technology and G¨oteborg University [1][2].

We used the Champman-Richards model to describe the yeast growth curve since this model was found to be the best to describe the growth pattern of the mutant and wild-type yeast strains under different environmental stresses [1]. For implementation purposes, the Champman-Richards model can be expressed as

$$v_t = \log(\frac{N_t}{N_0}) = \beta_0[1 - \beta_1 e^{-\beta_2 t}]^{\frac{1}{1-\beta_3}} \quad (3.1)$$

The parameters for the Champman-Richards model are set as the following based on the literature: β_0, $\beta_2 > 0$, $_3 > 1$, and $\beta_1 < 1$ - β_3 or β_0,$\beta_2 > 0$, $0 < \beta_3 < 1$, and 1 - $\beta_3 < \beta_1 < 1$.
Several with physically meaningful values can be expressed by using the "tunable lower-level" parameters ($\beta_0, \beta_1, \beta_2, \beta_3$) as the following:

- $A_z = \beta_0$; A_z is the asymptotic maximum value for which the yeast growth reaches (on the log scale).

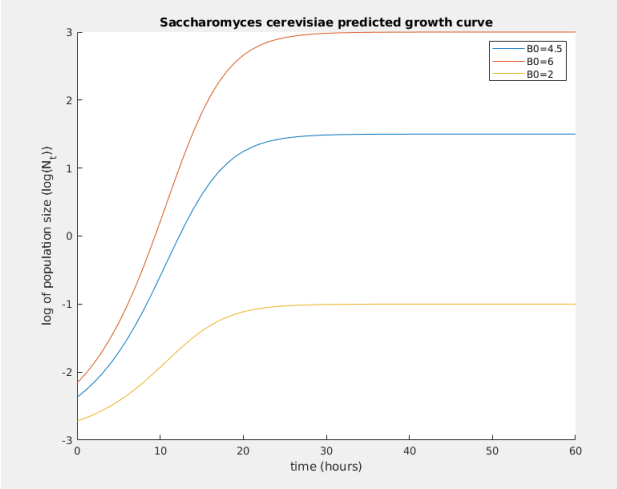
- Growth Rate ($growthrate$) = $\beta_0 * \beta_2 * ((\beta_3)^(\beta_3/(1-\beta_3)))$; this represents the max relative population growth rate of a given yeast population.

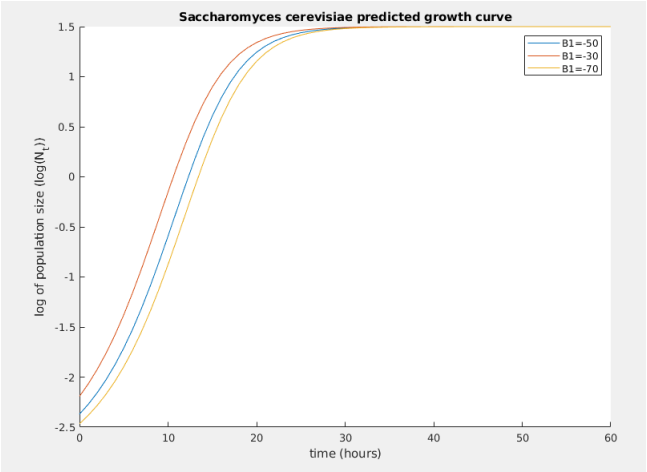
- Lag Time (lag) = $(\beta_0(1-\beta_1)^(1/(1-\beta_3)) - \beta_0 * \beta_3^(1/(1-\beta_3)) + growthrate * (log(\beta_1/(1-\beta_3))/\beta_2))/growthrate$; this represents the time for a given colony of yeast to adapt to their surrounding environments before initiating the exponential growth.

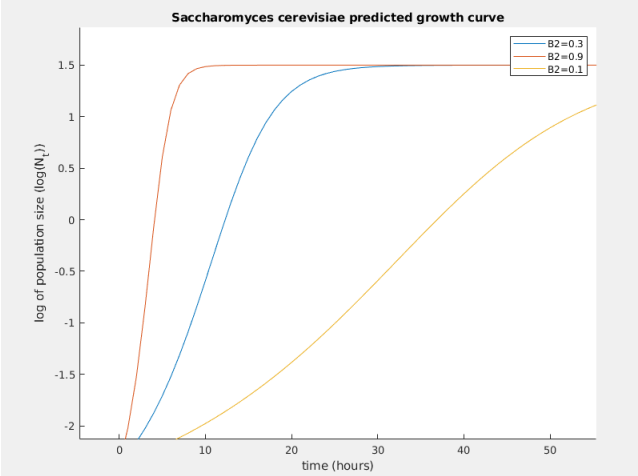
- Inflection time point ($time_I$) = $(log(\beta_1/(1-\beta_3)))/\beta_2$; this represents the inflection time point.

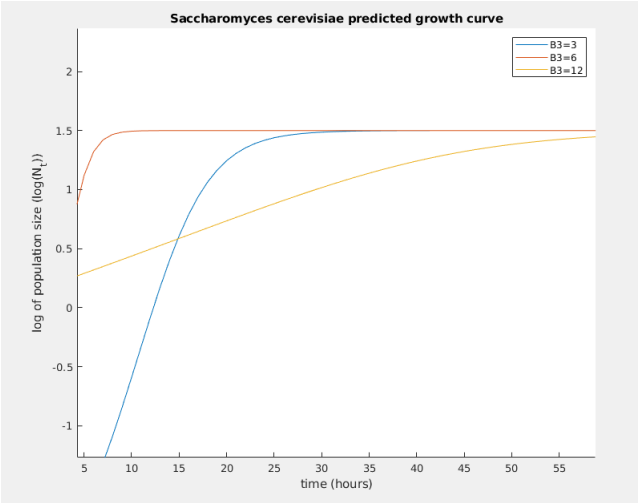
Based on the relationship between the physically meaningful values and the "tunable lower-level" parameters, we are able to solve for the "tunable lower-level" parameters based on the experimental results that are obtained through the experiments.

In the following graphs, we ran several simulations with different "tunable lower-level" parameters.









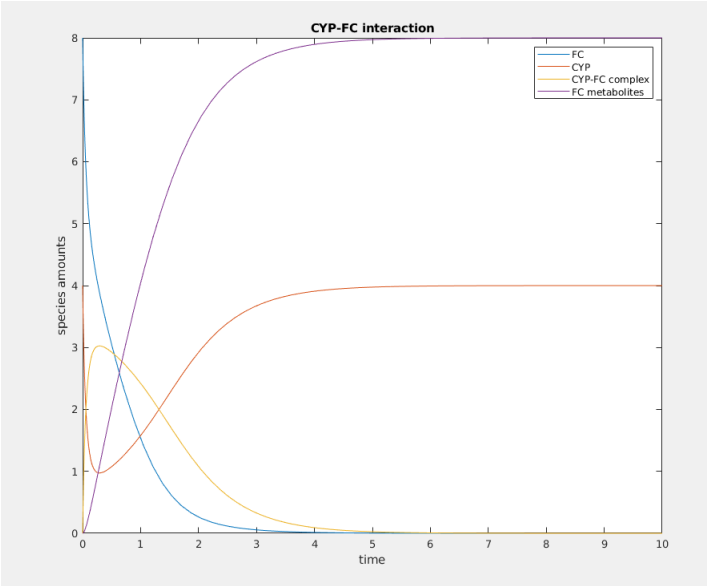
4 CYP, CYP Reductase, and FC interaction

The interactions among the CYP, CYP Reductase, and FC are modeled through the Michaelis–Menten kinetics which can be expressed as the following [3]:

$$\mathrm{E} + \mathrm{S} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} [\mathrm{ES}] \xrightarrow{k_2} \mathrm{P} + \mathrm{E} \quad (4.1)$$

- E: enzyme (in our model, it is the CYP)
- S: substrate (in our model, it is the FC)
- P: product (in our model, it is the FC metabolites)
- ES: enzyme/substrate complex
- k_1: binding rate of E and S
- k_{-1}: unbinding rate of ES
- k_2: transformation rate of ES

The graph for interaction with a limited amount of CYP and FC will behave as the following:

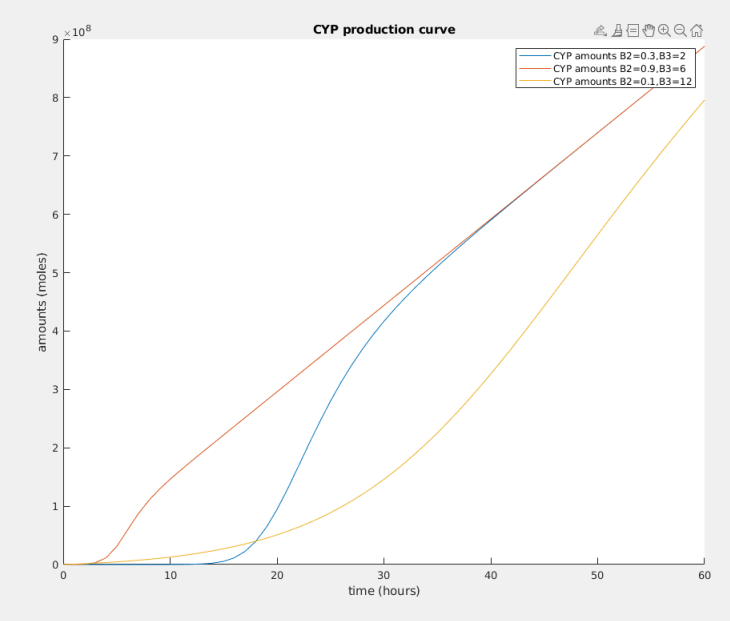


The interaction model that involves a CYP-producing yeast colony is based on the following important assumptions:

- The engineered yeasts follow the standard yeast growth curve.
- The plasmid burden on the engineered yeast colony is negligible.
- We assume existing CYP will not affect the future CYP production rate within the given time frame.
- We assume CYP are not self-degrading within the given time frame

By incorporating the Saccharomyces cerevisiae growth model with various "tunable lower-level" parameters and the CYP-CYP Reductase-FC interaction into our simulations, we are able to generate estimations for amounts of CYP that depends on the time upon the Saccharomyces cerevisiae starts growing in an environment with FC.

Moreover, based on the graphs from section 3 (east Growth Curve Modeling), in order to achieve optimal growth and potential CYP production. We may want to have greater B0, greater B1, and greater B2. This can be confirmed by the following graph shown below regarding the production of CYP based on the yeast colony growth curve. The ideal CYP production will then be quite similar to the one represented by the red curve as shown in the graph shown below.



5 Reference

[1] Asaduzzaman, M. (2007). Standardization of yeast growth curves from several curves with different initial sizes. Department of Mathematical Sciences. Chalmers University of Technology and Goteborg University. Sweden.

[2] Pylvänäinen, I. (2005). A parametric approach to yeast growth curve estimation and standardization. Chalmers Tekniska Hogskola (Sweden).

[3] MathWorks. (2021). Define Reaction Rates with Enzyme Kinetics.