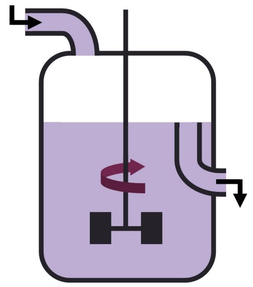
Evaluating Mathematical Models for Mixed-Substrate Bacterial Growth in a Chemostat

Aishani Sinha[1], Freya Bull[2]

[1]UCL Department of Computer Science, [2]UCL Department of Mathematics

Introducing the Chemostat



A chemostat is a well-mixed, continuously diluted volume contained within a vessel, in which growth substrate is externally supplied from a reservoir at a constant flow rate, and from which a mixture of bacteria and spent substrate is removed at the same rate, maintaining a constant volume within. The steady state population in the chemostat is reached when the rate of bacterial growth is equal to the dilution rate, creating an environment in which bacteria grow at exactly the rate at which they are removed.

Motivation

Models exist that effectively capture the dynamics of single substrate growth in a chemostat, which are of great practical importance in industries like pharmaceutical production.

However, in nature, it is rare for nutrients for to be present in the environment as a singular substrate – more often, bacteria will grow on a mixture of different substrates. Hence, there is a need for a model that effectively describes the dynamics of mixed-substrate bacterial growth.

Existing Single Substrate Model

The Monod model describes the bacterial growth rate, μ, as a function of the singular substrate concentration, s, where K_s is a saturation constant and μ_{max} is the maximum growth rate:

$$\boldsymbol{\mu(s) = \frac{\mu_{max} s}{s + K_s}}$$

Ordinary Differential Equations can be written to model single-substrate bacterial in a chemostat, which lead to the steady-state solutions for the growth rate, substrate concentration and bacterial concentration respectively:

$$\boldsymbol{\tilde{\mu} = D, \tilde{s} = \frac{K_s D}{\mu_m - D}, \tilde{\rho} = Y\left(s_0 - \frac{K_s D}{\mu_m - D}\right)}$$

where D is the dilution rate, and Y is the yield coefficient.

Bacterial Growth Rate Models

Below are mixed-substrate bacterial growth rate models. The higher-order models in (3), (4) and (5) below account for interactions between substrates during bacterial growth.

(1) Sequential Uptake Model

The first and simplest multi-substrate bacterial growth model assumes that bacteria simply switch substrate once they have depleted one enough that another becomes more favourable. This means that the overall steady-state bacterial concentration is equal to the sum of that of each substrate, as below:

$$\boldsymbol{\widetilde{\rho_{total}} = \sum_{i=1}^{n} Y_i\left(s_{0;i} - \widetilde{s_i}\right)}$$

(2) Lendenmann Ratio Model

In 1996, Lendenmann proposed a mixed-substrate model where the steady-state concentrations of individual substrates in the chemostat were proportional to the ratio of the input substrate in the overall growth medium. Assuming Monodic growth on a single substrate, this can be expressed as:

$$\boldsymbol{\widetilde{s_1} = \frac{K_s D}{\mu_m - D} \cdot \frac{s_{0;1}}{\sum_{i=1}^{n} s_{0;i}}}$$

(3) Saturation-Based Interaction Model (Yoon)

This model incorporates interaction parameters that account for the competitive inhibition effect that each substrate exhibits on the utilisation of the other substrates, and is given by the ratio of saturation constants:

$$\boldsymbol{\mu(s1, s2) = \frac{\mu_{m;1} s_1}{K_{s;1} + s_1 + \frac{K_{s;1}}{K_{s;2}} s_2} + \frac{\mu_{m;2} s_2}{K_{s;2} + s_2 + \frac{K_{s;2}}{K_{s;1}} s_1}}$$

(4) Extended Interaction Model 1

This model expresses interactions between substrates in an even higher-order manner, through introducing a term of $s_1 s_2$ into the growth rate equation:

$$\boldsymbol{\mu(s1, s2) = \frac{\mu_{m;1} s_1}{\left(K_{s;1} + s_1\right)\left(1 + \frac{s1}{K_{s;2}}\right)} + \frac{\mu_{m;2} s_2}{\left(K_{s;2} + s_2\right)\left(1 + \frac{s1}{K_{s;1}}\right)}}$$

(5) Extended Interaction Model 2

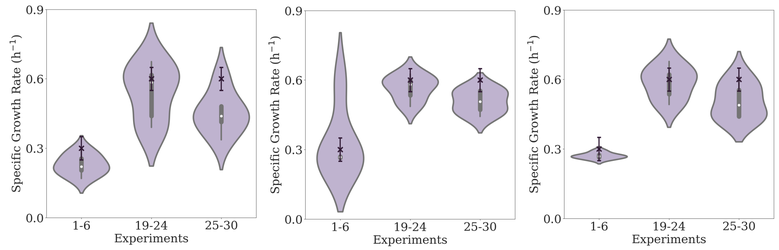
As above, this model also incorporates an $s_1 s_2$ term in the equation, but neglects the term $\frac{K_{s;i}}{K_{s;j}} s_i$ on the denominator:

$$\boldsymbol{\mu(s1, s2) = \frac{\mu_{m;1} s_1}{K_{s;1} + s_1\left(1 + \frac{s1}{K_{s;2}}\right)} + \frac{\mu_{m;2} s_2}{K_{s;2} + s_2\left(1 + \frac{s_1}{K_{s;1}}\right)}}$$

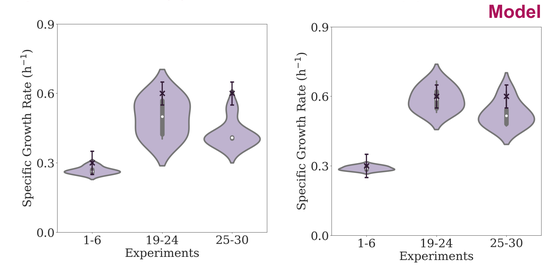
Quantitative Comparison of Models

Knowing that $\boldsymbol{\tilde{\mu} = D}$ in steady-state, we can calculate this specific bacterial growth rate using experimental data for each of the five models. We can then compare these theoretical values with the actual dilution rate used in the experiments to evaluate the validity of the models.

We produced violin plots to compare the actual dilution rate used in each experiment, shown by the black cross with error bars, to the distribution of the theoretical calculations for the specific bacterial growth rate.



(a) Sequential Uptake Model (b) Lendenmann Model (c) Saturation-Based Yoon Model



(d) Extended Interaction Model 1 (e) Extended Interaction Model 2

In all cases, the actual dilution rate falls within the bounds of the distribution of the theoretically calculated values for the specific growth rate, but upon analysing the sum of square deviations between the actual dilution rate and theoretical values, the ***Extended Interaction Model 2*** outperforms the rest.

Conclusion

The results suggest that the higher-order interactive models, specifically the ***Extended Interaction Model 2***, are generally better in predicting the specific growth rate in steady-state conditions, highlighting the complexity of the underlying mechanisms governing mixed-substrate bacterial growth.

These higher order multi-substrate growth modes can be thought of as corrective terms to the single-substrate growth mode; giving rise to different bounds on the existing single substrate steady state. In the future, we would like to extend this model to account for even higher-order interactions between individual substrates.

[1]aishani.sinha.23@ucl.ac.uk, [2]f.bull@ucl.ac.uk

Urs Lendenmann and Thomas Egli (July 1998). Kinetic models for the growth of Escherichia coli with mixtures of sugars under carbon-limited conditions. Biotechnology and Bioengineering
H. Yoon, G. Klinzing, and H. W. Blanch (August 1977) Competition for mixed substrates by microbial populations. Biotechnology and Bioengineering
Jacques Monod (1942) Recherches sur la croissance des cultures bacte'riennes (Studies on the growth of bacterial cultures)
Gideon Okpokwasili and C.O. Nweke (February 2006) Microbial growth and substrate utilization kinetics. African Journal of Biotechnology