KINETICS CORRESPONDING TO THE GROWTH OF MYCOBACTERIUM TUBERCULOSIS IN VITRO UNDER DIFFERENT PHYSICAL METHODS OF IDENTIFICATION

Eugene B. Postnikov[1], Anastasia I. Lavrova[2,3], Olga A. Manicheva[2]

Научно-исследовательский центр физики конденсированного состояния
Research Center for Condensed Matter Physics
Санкт-Петербургский государственный университет
Медицинский факультет

[1]*Dept. of Theoretical Physics, RCCMP, Kursk State University*
[2]*St Petersburg Research Institute of Phthisiopulmonology,*
[3] *Medical Faculty, St Petersburg State University*

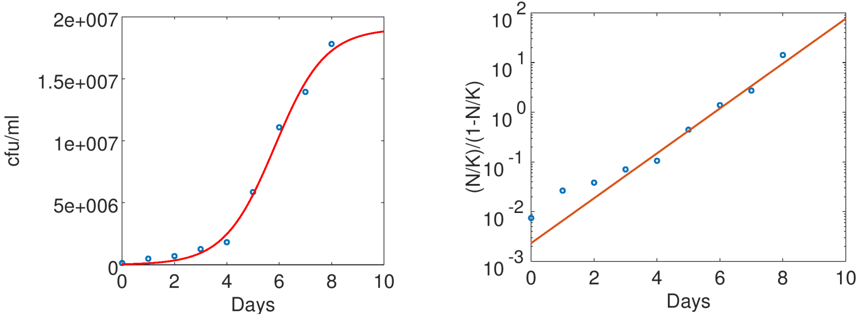
Methods of population size estimations

In the case of ***Mycobacterium tuberculosis*** **bacteria**:

Direct colony-forming units (cfu) counting	Most accurate numbers	Very labour-intensive procedure; contagious procedure
Optical densimetry (OD600)	May be automated, there is a correspondence to cfu	Not a simple automatization; coarse time steps; contagious procedure
BACTEC Kit	Fast and accurate automated procedure	A direct correspondence to cfu is unknown
Resazurin assay	Fast automated procedure	Disputable correspondence to cfu; poor quantization of data

Optical density at 600 nm

Logistic growth $N = \frac{K}{1+\exp[-r(t-t_m)]}$ after a transient regime:



OD600 units recalculated into cfu using the correlation given in K. Peñuelas-Urquides et al. Measuring of Mycobacterium tuberculosis growth. A correlation of the optical measurements with colony forming units *Braz J Microbiol.* **44** 2013 287289.

BD BACTEC™ MGIT™

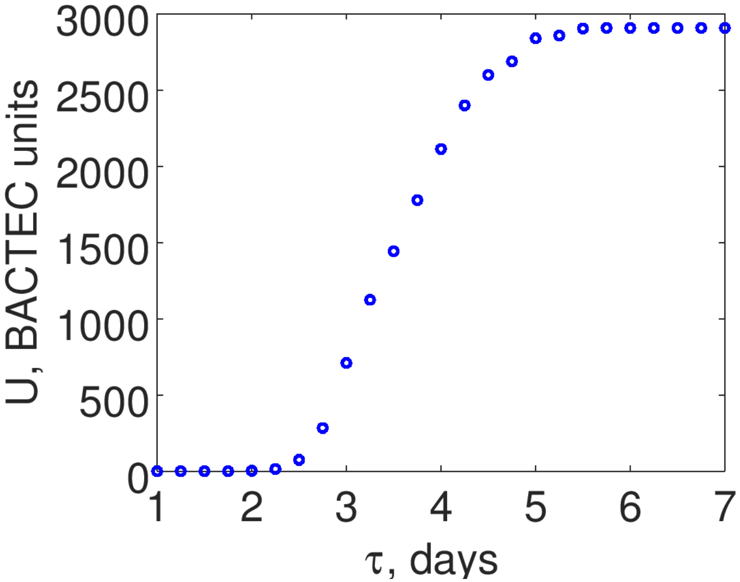
Basic principles:
Mycobacteria Growth Indicator Tube contains a modified Middlebrook 7H9 Broth, which supports the growth and detection of mycobacteria

Source: `https://www.bd.com`

The fluorescent compound is sensitive to the presence of oxygen dissolved in the broth:

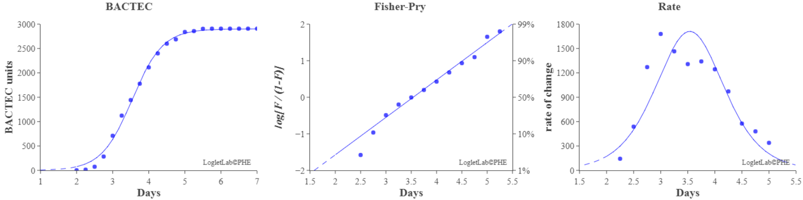
- the initial concentration of dissolved oxygen quenches the emission from the compound;
- actively growing and respiring microorganisms consume the oxygen which allows the compound to fluoresce.

A typical BACTEC curve

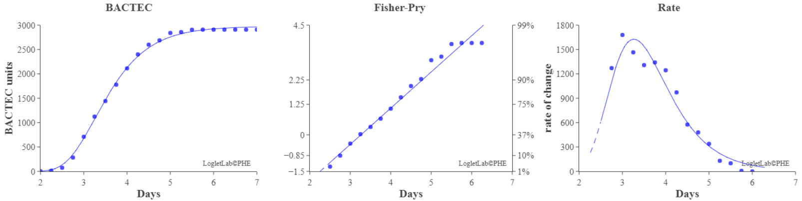


First trials: classic sigmoids

The Verhulst (logistic) growth:

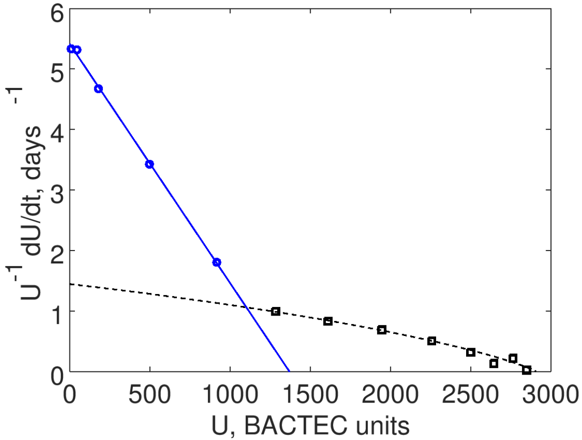


The Gompertz growth:



A DETAILED PHASE PLANE ANALYSIS

$$\frac{dU_1}{U_1 dt} = r\left(1 - \frac{U_1}{K}\right)$$



$$\frac{dU_2}{U_2 dt} = \left[k_1 - k_2 \log\left(1 - \frac{U_2}{U_{max}}\right)\right]\left(1 - \frac{U_2}{U_{max}}\right)$$

An analogy with one of demographic models

The second stage of the growth

$$\frac{1}{U_2}\frac{dU_2}{dt} = \left[k_1 - k_2 \log\left(1 - \frac{U_2}{U_{max}}\right)\right]\left(1 - \frac{U_2}{U_{max}}\right),$$

is a particular case of [ ]

$$\frac{dU_2}{dt} = U_2\left[a\mu + bU_2 \cdot {}_2F_1\left(1, \frac{a}{c}; 1 + \frac{a}{c}; \lambda U_2^{\frac{c}{a}}\right) + \right]\left(1 - \lambda U_2^{\frac{c}{a}}\right),$$

for $a = c$, when the hypergeometric function

$${}_2F_1(1, 1, 2; z) = -z^{-1}\log(1 - z).$$

EBP, Analytical properties of a three-compartmental dynamical demographic model. *Physical Review E* **92** (2015) 012718

A three-compartmental BACTEC model

$$\frac{dU_2}{dt} = aU_2S(1-N), \quad (1)$$
$$\frac{dS}{dt} = bU_2S, \quad (2)$$
$$\frac{dN}{dt} = aSN(1-N), \quad (3)$$

1. Fluorescence induced by the consumed oxygen and limited by the presence of bacteria
2. Oxigen consumed by bacteria
3. Bacterial logistic growth supported by the consumed oxygen

Invariants-based reduction:

$$\frac{dU_2}{dN} = \frac{U_2}{N} \Rightarrow N = \frac{U_2}{U_{max}}; \quad \frac{dU_2}{dt} = aU_2S\left(1-\frac{U_2}{U_{max}}\right)$$

$$\frac{dU_2}{dS} = \frac{a}{b}\left(1-\frac{U_2}{U_{max}}\right) \Rightarrow$$

$$aS = \left[aS(0) + bU_{max}\log\left(1-\frac{U_2(0)}{U_{max}}\right)\right] - bU_{max}\log\left(1-\frac{U_2}{U_{max}}\right)$$

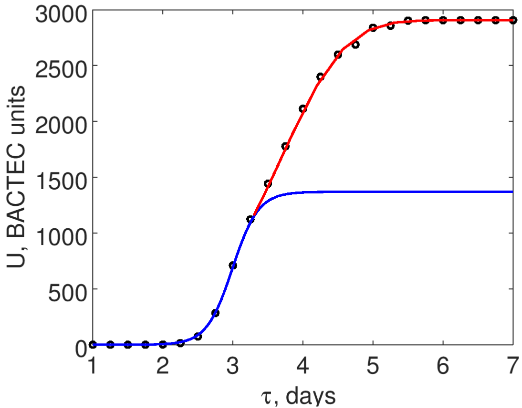
TWO-STAGE MODEL SOLUTION *vs.* DATA

$$U = U_1 \cup U_2$$

$$\frac{dU_1}{dt} = rU_1\left(1 - \frac{U_1}{K}\right)$$

$$U_1^{(end)} \approx 0.84K,$$

$$U_2(0) \cong 1.1U_1^{(end)}$$



$$\frac{dU_2}{dt} = U_2\left[k_1 - k_2 \log\left(1 - \frac{U_2}{U_{max}}\right)\right]\left(1 - \frac{U_2}{U_{max}}\right)$$

Growth rate regulation

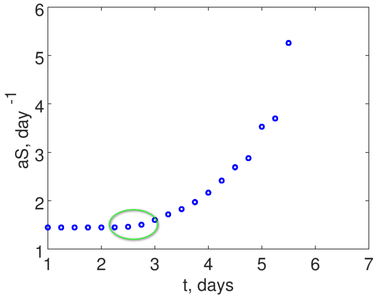
$$aS \approx aS(0) - bU_{max} \log\left(1 - \frac{U}{U_{max}}\right)$$

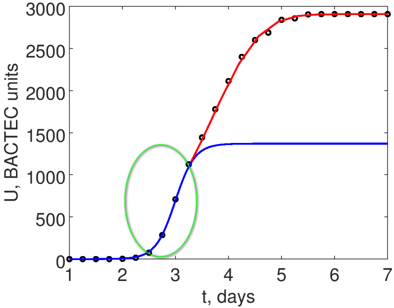
Reduction of

$$\frac{dU_2}{dt} = aU_2S(1-N),$$
$$\frac{dS}{dt} = bU_2S,$$
$$\frac{dN}{dt} = aSN(1-N),$$

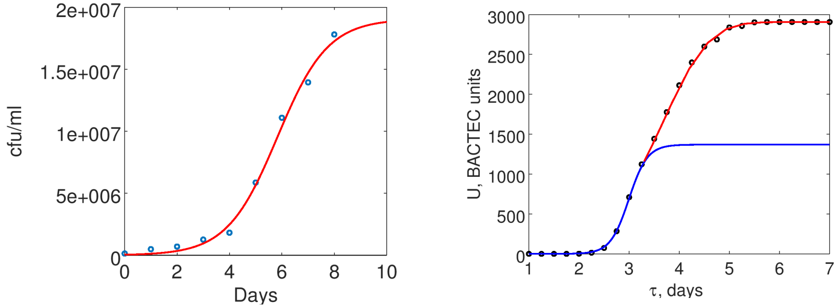
for $S = S_0 = \text{const}$:

$$\frac{dU_2}{dt} = aS_0U_2(1 - U_2/U_{max}),$$
$$\frac{dN}{dt} = aS_0N(1-N);\ N = \frac{U_2}{U_{max}}$$





BACTEC *vs.* OD600 GROWTH RATES



$r = 1.04\,\text{day}^{-1}$; $t^{OD}_{0.95\max(N)} \approx 8.6$ days

$aS(3.5) = 1.83\,\text{day}^{-1}$; $t^{B}_{0.95\max(U)} \approx 4.8$ days

$$\frac{t^{OD}_{0.95}}{t^{B}_{0.95}} \cong 1.79; \qquad \frac{aS(3.5)}{r} \cong 1.75$$

Conclusion

The main inference: dynamics of BACTEC fluorescence may be explained by the model, which takes into account the principal oxigen-based coupling between bacteria density and fluorescence magnitude.

Corollary: *a recipe for estimating the actual bacteria growth rate*

- draw the phase portrait of BACTEC fluorescence data ($U^{-1}dU/dt = f(U)$);
- determine the beginning of a transition from initial transient regime to the oxigen-reducing regime;
- find the growth rate corresponding to this beginning state;
- rescale this growth rate to the value of the reference cfu growth rate under “natural environment conditions”

Thank you for attention!