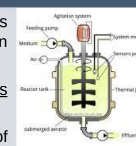
FE 411 FOOD BIOTECHNOLOGY

2-Microbial Kinetics

Dr. Ali Coşkun DALGIÇ

INTRODUCTION



- Understanding the growth kinetics of microbial cells is important for the design and operation of fermentation systems employing them.
- Cell kinetics deals with the rate of cell growth and how it is affected by various chemical and physical conditions.
- Unlike enzyme kinetics, cell kinetics is the result of numerous complicated networks of biochemical and chemical reactions and transport phenomena, which involves multiple phases and multicomponent systems.
- The heterogeneous mixture of young and old cells is continuously changing and adapting itself in the media environment which is also continuously changing in physical and chemical conditions. As a result, accurate mathematical modeling of growth kinetics is impossible to achieve.

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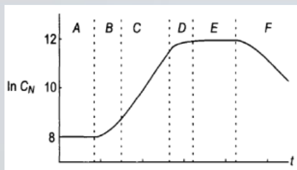
Understanding cell kinetics: the basics	Modes of cell cultivations
Cell growth and cell division	Batch operation
Substrate consumption	Continuous operation
Product formation	Fed-batch operation
Environmental effects on cell kinetics	

CELL GROWTH in A BATCH REACTOR CULTURE

- Closed system
- Constant volume
- Limited nutrient

GROWTH CYCLE FOR BATCH CULTIVATION

If you inoculate unicellular microorganisms into a fresh sterilized medium and measure the cell number density with respect to time and plot it, you may find that there are six phases of growth and death.



A. Lag phase
B. Accelerated growth phase
C. Exponential growth phase
D. Decelerated growth phase
E. Stationary phase
F. Death phase

GROWTH CYCLE FOR BATCH CULTIVATION

A. Lag phase: A period of time when the change of cell number is zero.

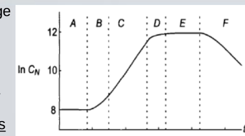
B. Accelerated growth phase: The cell number starts to increase and the division rate increases to reach a maximum.

C. Exponential growth phase: The cell number increases exponentially as the cells start to divide. The growth rate is increasing during this phase, is constant at its maximum value.

D. Decelerated growth phase: After the growth rate reaches a maximum, it is followed by the deceleration of both growth rate and the division rate.

E. Stationary phase: The cell population will reach a maximum value and will not increase any further.

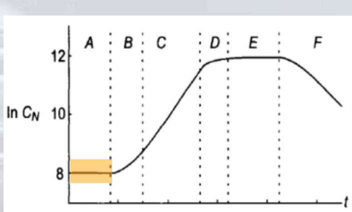
F. Death phase: After nutrients available for the cells are depleted, cells will start to die and the number of viable cells will decrease.



GROWTH CYCLE FOR BATCH CULTIVATION

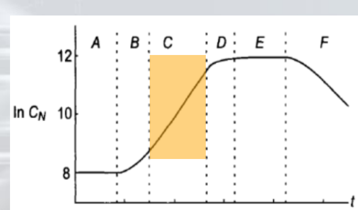
1. Lag phase: The lag phase (or initial stationary, or latent) is an initial period of cultivation during which the change of cell number is zero or negligible. Even though the cell number does not increase, the cells may grow in size during this period.

The length of this lag period depends on many factors such as the type and age of the microorganisms, the size of the inoculum, and culture conditions.



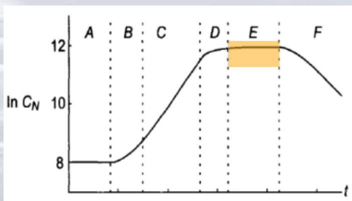
GROWTH CYCLE FOR BATCH CULTIVATION

2. Exponential phase: In unicellular organisms, the progressive doubling of cell number results in a continually increasing rate of growth in the population. A bacterial culture undergoing balanced growth mimics a first-order autocatalytic chemical reaction.



GROWTH CYCLE FOR BATCH CULTIVATION

3. Stationary phase: The growth of microbial populations is normally limited either by the exhaustion of available nutrients or by the accumulation of toxic products of metabolism. As a consequence, the rate of growth declines and growth eventually stops. At this point a culture is said to be in the stationary phase.



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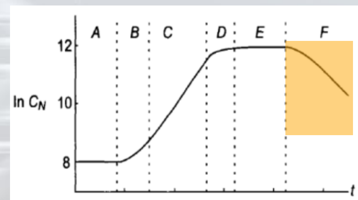
GROWTH CYCLE FOR BATCH CULTIVATION

4. Death phase: The stationary phase is usually followed by a death phase in which the organisms in the population die. Death occurs either because of the depletion of the cellular reserves of energy, or the accumulation of toxic products. Like growth, death is an exponential function. In some cases, the organisms not only die but also disintegrate, a process called lysis.



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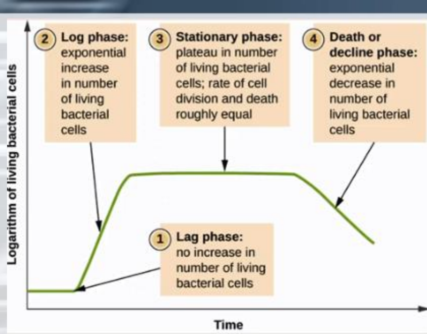
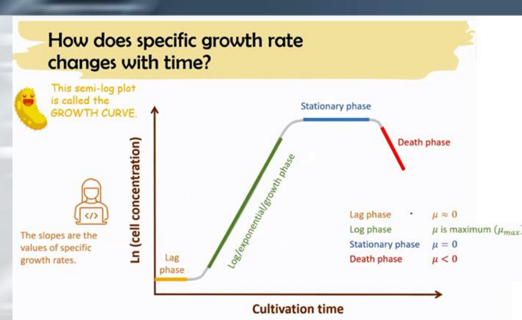
GROWTH CYCLE FOR BATCH CULTIVATION





Kinetic of cell growth

Cells' growth kinetic =(how fast)they grow & what governs it.

$$\boxed{\frac{dx}{dt} = \mu x}$$

μ is not always a constant. They changes with time and depends on what controls the growth mechanisms of the cells.

μ = specific growth rate (per unit time)
x = cell concentration at a given time (g/L)

To understand the growth kinetics, one needs to understand the specific growth rate.

How does specific growth rate changes with cultivation time?

In the simplest analysis;

$$\frac{dx}{dt} = \mu x$$

$$\int_{x_0}^{x} \frac{1}{x} dx = \int_{0}^{t} \mu \, dt$$

If μ is a constant.

$$\ln x - \ln x_0 = \mu t$$

$$\boxed{\ln x = \mu t + \ln x_0}$$

μ is the slope of a semi-log plot.

This linear transformation allows the plot in linear graph.

$$\ln x = \mu t + \ln x_0$$

$$y = mx + c$$

So ... the slope from a plot of $\ln x$ versus time would tell us the μ values.

Specific growth rate, μ

2 ways to look at it.

Numeric values

- Determine specific growth rate at a given time.
- Often determine from experimental data.

Mathematical terms

- Attempt to identify effects of factors on μ value.

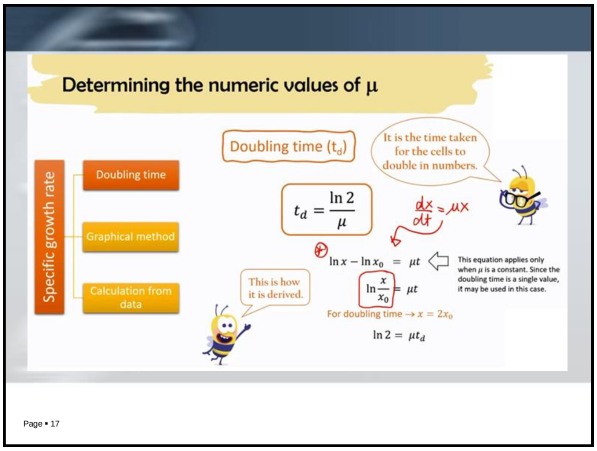
Involve fitting of the kinetic equation to experimental data to prove the effects.

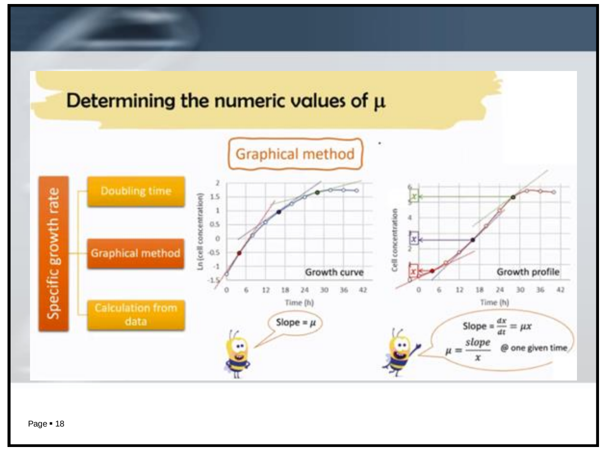
Determining the numeric values of μ

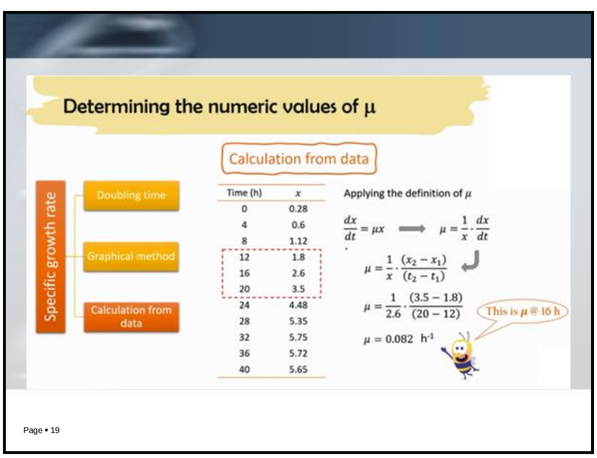
We sometimes call them "instantaneous μ"

Specific growth rate

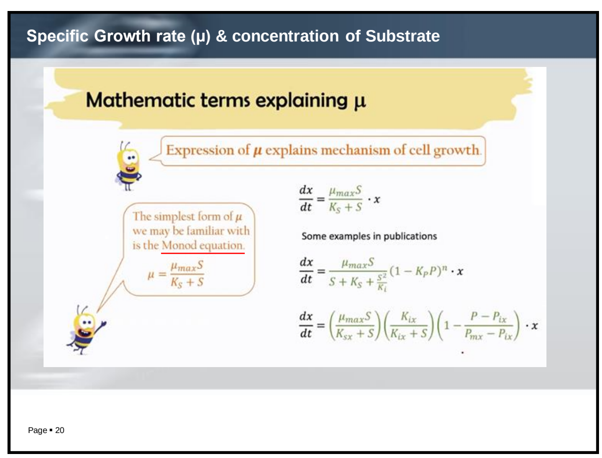
- **Doubling time**
  - Time taken for cells to increase twice in number.
  - Use when there was data on changes in number of cells over time.
- **Graphical method**
  - Determine from the slopes of a growth curve.
  - May calculate from a growth profile, following the definition of μ.
- **Calculation from data**
  - Calculation from the definition of μ using the experimental data.







Time (h)	x
0	0.28
4	0.6
8	1.12
12	1.8
16	2.6
20	3.5
24	4.48
28	5.35
32	5.75
36	5.72
40	5.65



Factors affecting the specific growth rate

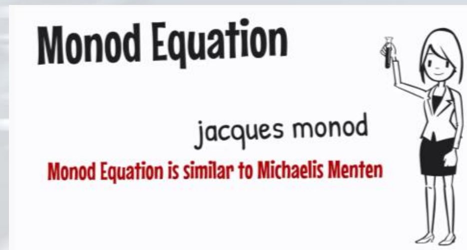
- ***Substrate Concentration:*** One of the most widely employed expressions for the effect of substrate concentration on μ is the *Monod equation*, which is an empirical expression based on the form of equation normally associated with enzyme kinetics :

$$\mu = \frac{\mu_{max} C_S}{K_S + C_S}$$

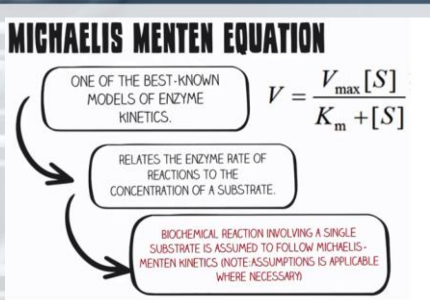
where C_s is the concentration of the limiting substrate in the medium and K_s is a system coefficient.

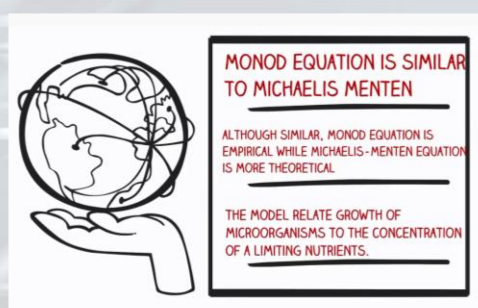
This equation has the same form as the rate equation for an enzyme catalyzed reaction, the Michaelis-Menten equation:

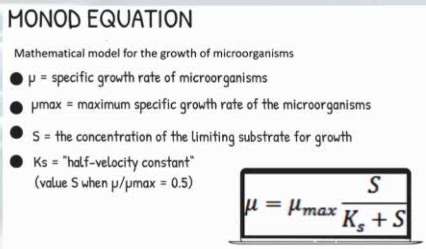
$$r_P = \frac{r_{max} C_S}{K_M + C_S}$$

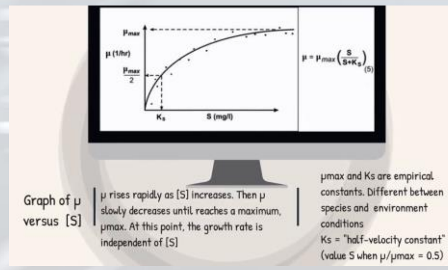


- Monod equation: empirical mathematical model
- French scientist Jacques Monod, Nobel Prize winner in 1965

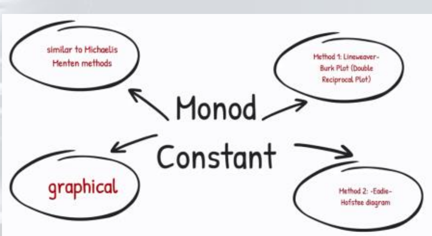








- The value of *Ks* is equal to the concentration of nutrient when the specific growth rate is half of its maximum value μ_{max}. According to the Monod equation, further increase in the nutrient concentration after μ reaches μ_{max} does not affect the specific growth rate.



EVALUATION OF KINETIC PARAMETERS

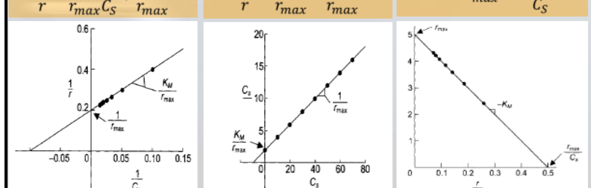
- The Michaelis-Menten equation can be rearranged to be expressed in linear form. This can be achieved in three ways:

$$r = \frac{r_{max} C_S}{K_M + C_S}$$

$$\frac{1}{r} = \frac{K_M}{r_{max} C_S} + \frac{1}{r_{max}}$$

$$\frac{C_S}{r} = \frac{K_M}{r_{max}} + \frac{C_S}{r_{max}}$$

$$r = r_{max} - r\frac{K_m}{C_S}$$



The Lineweaver-Burk plot | *The Langmuir plot* | *The Eadie-Hofstee plot*

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Method 1 : lineweaver Plot

(double reciprocal plot)

Described by Hans Lineweaver and Dean Burk in 1934 for enzyme kinetics

Lineweaver Burke Plot Method

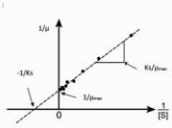
$$\mu = \mu_{max}\frac{S}{K_s + S}$$

Taking the reciprocal of the above Equation, we will obtain

$$\frac{1}{\mu} = \frac{K_s}{\mu_{max}[S]} + \frac{1}{\mu_{max}} \quad \text{Equation 2}$$

Lineweaver Burke Plot Method

$$\frac{1}{\mu} = \frac{K_s}{\mu_{max}[S]} + \frac{1}{\mu_{max}} \quad \text{Equation 2}$$



Plotting 1/μ against 1/[S] will give us a straight line graph

slope = Ks/μmax.
x intercept = -1/Ks
y intercept = 1/μmax

Method 2 : Eadie Hofstee Diagram

Woolf-Eadie-Augustinsson-Hofstee or Eadie-Augustinsson plot

-linear plot
-better than Lineweaver Plot because it gives equal weight to data points in any range of substrate concentration or reaction rate.

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Eadie Hofstee Diagram

$$\mu = \mu_{max}\frac{S}{K_s + S}$$

Invert and multiply with μmax. Then rearrange to get

$$\mu_{max} = \frac{vK_s+\mu[S])}{[S]} = \frac{\mu K_s}{[S]} + \mu$$

isolate μ to yield

$$\mu = -K_s\frac{\mu}{[S]} + \mu_{max} \quad \text{Equation 3}$$

Eadie Hofstee Diagram

$$\mu = -K_s\frac{\mu}{[S]} + \mu_{max}$$

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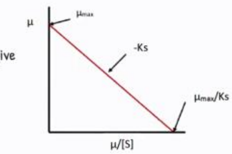
Plotting μ against μ/[S] will give us a straight line

slope=-Ks,
y intercept =μmax
x intercept = μmax/Ks



Method 3 : The Langmuir plot

The Langmuir plot

The Langmuir Plot Method

$$\mu = \mu_{max}\frac{S}{K_s + S}$$

Taking the reciprocal of the above Equation, we will obtain

$$\frac{1}{\mu} = \frac{K_s}{\mu_{max}[S]} + \frac{1}{\mu_{max}} \quad \text{Equation 2}$$

Multiply with [s]

$$\frac{[S]}{\mu} = \frac{K_s}{\mu_{max}} + \frac{[S]}{\mu_{max}} \quad \text{Equation 3}$$

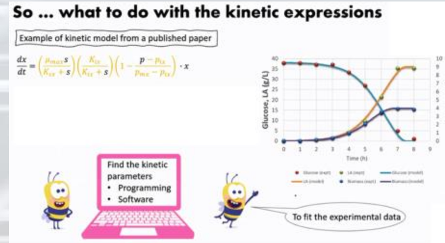
Factors affecting the specific growth rate

- ***Product Concentration:*** As cells grow they produce metabolic byproducts which can accumulate in the medium. The growth of microorganisms is usually inhibited by these products, whose effect can be added to the Monod equation as follows:

$$\mu = \mu_{\max} \left(\frac{C_S}{K_S + C_S} \right) \left(\frac{K_p}{K_p + C_p} \right)$$

- ***Other conditions:*** The specific growth rate of microorganisms is also affected by medium pH, temperature, and oxygen supply. The optimum pH and temperature differ from one microorganism to another.

So ... what to do with the kinetic expressions



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Modeling of the Bacterial Growth Curve

M. H. ZWIETERING,* I. JONGENBURGER, F. M. ROMBOUTS, AND K. VAN 'T RIET
APPLIED AND ENVIRONMENTAL MICROBIOLOGY, June 1990, p. 1875-1881

Several sigmoidal functions (logistic, Gompertz, Richards, Schnute, and Stannard) were compared to describe a bacterial growth curve. They were compared statistically by using the model of Schnute, which is a comprehensive model, encompassing all other models. The *t* test and the *F* test were used. With the *t* test, confidence intervals for parameters can be calculated and can be used to distinguish between models. In the *F* test, the lack of fit of the models is compared with the measuring error. Moreover, the models were compared with respect to their ease of use. All sigmoidal functions were modified so that they contained biologically relevant parameters. The models of Richards, Schnute, and Stannard appeared to be basically the same equation. In the cases tested, the modified Gompertz equation was statistically sufficient to describe the growth data of *Lactobacillus plantarum* and was easy to use.

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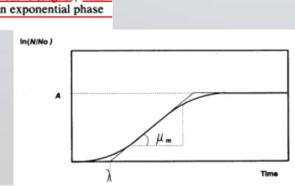
Bacterial growth often shows a phase in which the specific growth rate starts at a value of zero and then accelerates to a maximal value (μ_m) in a certain period of time, resulting in a lag time (λ). In addition, growth curves contain a final phase in which the rate decreases and finally reaches zero, so that an asymptote (A) is reached. When the growth curve is defined as the logarithm of the number of organisms plotted against time, these growth rate changes result in a sigmoidal curve (Fig. 1), with a lag phase just after $t = 0$ followed by an exponential phase and then by a stationary phase.



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Model	Equation	Modified equation[a]
Logistic	$y = \frac{a}{[1 + \exp(b - cx)]}$	$y = \frac{A}{\left\{1 + \exp\left[\frac{4\mu_m}{A}(\lambda - t) + 2\right]\right\}}$
Gompertz	$y = a \cdot \exp[-\exp(b - cx)]$	$y = A\exp\left\{-\exp\left[\frac{\mu_m \cdot e}{A}(\lambda - t) + 1\right]\right\}$
Richards	$y = a\,\{1 + v \cdot \exp[k(\tau - x)]\}^{(-1/v)}$	$y = A\left\{1 + v \cdot \exp(1 + v) \cdot \exp\left[\frac{\mu_m}{A} \cdot (1 + v)\left(1 + \frac{1}{v}\right) \cdot (\lambda - t)\right]\right\}^{(-1/v)}$
Stannard	$y = a\left\{1 + \exp\left[-\frac{(l + kx)}{p}\right]\right\}^{(-p)}$	$y = A\left\{1 + v \cdot \exp(1 + v) \cdot \exp\left[\frac{\mu_m}{A} \cdot (1 + v)\left(1 + \frac{1}{v}\right) \cdot (\lambda - t)\right]\right\}^{(-1/v)}$
Schnute	$y = \left\{y_1^b + \left(y_2^b - y_1^b\right) \cdot \frac{1 - \exp[-a(t - \tau_1)]}{1 - \exp[-a(\tau_2 - \tau_1)]}\right\}^{1/b}$	$y = \left(\mu_m \frac{(1 - b)}{a}\right)\left[\frac{1 - b \cdot \exp(a \cdot \lambda + 1 - b - at)}{1 - b}\right]^{1/b}$

[a] $e = \exp(1)$.

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