EB – 12 Homogeneous Reactions

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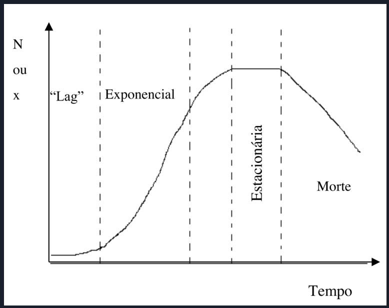
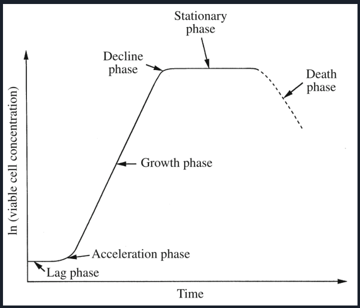
Conteúdo

12.8 Bioreactor Kinetics

12.8.1 Batch Growth

When cells are grown in batch culture, several phases of cell growth are observed. The different phases of growth are more readily distinguished when the logarithm of viable cell concentration is plotted against time





Phase	Specific growth rate	Description
Lag	$\mu \approx 0$	Cells adapting to new environment
Acceleration	$\mu < \mu_{\max}$	Cell population starts growing
Growth	$\mu \approx \mu_{\max}$	Growth achieve maximum rate
Decline	$\mu < \mu_{\max}$	Culture reaches limitant in nutrients or build-up of products
Stationary	$\mu = 0$	Cell death and birth equalize
Death	$\mu < 0$	Cells dying faster than they can multiply

Growth rate: $r_X = \frac{\mathrm{d}x}{\mathrm{d}t} = \mu\, x$

Growth rate

The cell growth rate (r_X) measures the change in volumetric concentration of viable cells (unit $(x) = \mathrm{g/m^3}$)

$$r_X = \frac{\mathrm{d}x}{\mathrm{d}t} = \mu\, x \implies \Delta \ln x = \mu\, t \tag{12.1}$$

$$\dim x = \mathrm{M/L^3}; \quad \dim \mu = \mathrm{T^{-1}}$$

Doubling time t_d Cell growth rates are often expressed in terms of the time it takes to duplicate the population.

$$x = 2\, x_0 : t = t_d = \frac{\log 2}{\mu} \tag{12.2}$$

$$t_d \implies \Delta \ln x = \ln \frac{x}{x_0} = \ln \frac{2\, x_0}{x_0} = \ln 2 = \mu\, t_d$$

12.8.2 Balanced Growth

In an environment favourable for growth, cells regulate their metabolism and adjust the rates of various internal reactions so that a condition of balanced growth occurs. During balanced growth, the composition of the biomass remains constant. For the biomass composition to remain constant during growth, the specific rate of production of each component in the culture must be equal to the cell specific growth rate μ.

In most cultures, balanced growth occurs at the same time as exponential growth.

$$\boldsymbol{r_P = \mu\, p} \tag{12.3}$$

Another point is that the cell consumption is also constant

$$\frac{\mathrm{d}\boldsymbol{x}}{\mathrm{d}\boldsymbol{t}} = \boldsymbol{r_X}$$

12.8.3 Effect of Substrate Concentration

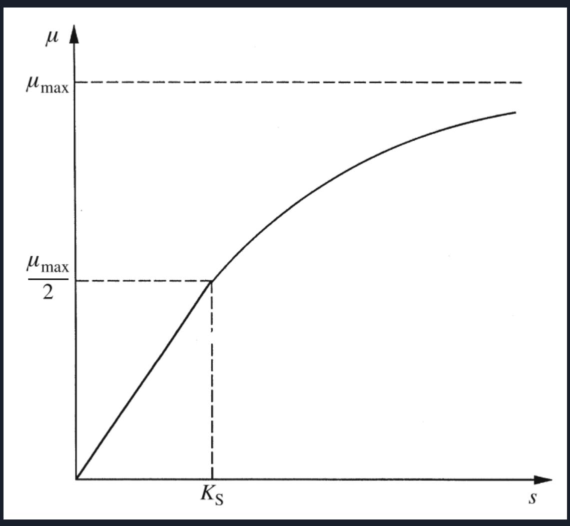
During the growth and decline phases of batch culture, the specific growth rate of the cells depends on the concentration of nutrientes in the medium.

Often a single substrate exerts a dominant influence on the rate of growth; this component is know as the growth-rate-limiting substrate (S).

During balanced growth, the specific growth rate is related to the concentration of the growth limiting substrate by a homologue of the *Michaelis–Menten* expression, the Monod equation:

$$\mu = \frac{\mu_{\max}\, s}{K_S + s} = \frac{\mu_{\max}}{1 + K_S/s} \tag{12.4}$$

$$\dim s = \frac{\mathrm{M}\ (\mathrm{S})}{\mathrm{L}^3}$$



Rate behavious based on concentration of substrate

if μ is dependent on the substrate concentration as indicated by the Monod equation, how can μ remain constant during the growth phase? Typical values for the substrate constant (K_S) are very small in order of mg/L for carbonhydrate substrates and µg/L for other compounds such as aminoacids.

The behaviour of specific growth rate (μ) with the concentration of substrate in relation to the substrate constant K_S follows:

Growth	$s \gtrsim K_S\, 10$	$\Longrightarrow \mu \approx \mu_{\max}$
Decline Start	$s \in K_S * [10, 1]$	$\Longrightarrow \mu = \mu_{\max}/(1 + K_S/s)$
Decline	$s \approx K_S$	$\Longrightarrow \mu \approx \mu_{\max}/2$
Decline End	$s < K_S$	$\Longrightarrow \mu = \mu_{\max}/(1 + K_S/s)$
Statinoary	$s \ll K_S$	$\Longrightarrow \mu \approx 0$

Some examples for the substrate constant K_S:

Microoragnism (genus)	Limiting Substrate	K_S/(mg/L)
Saccgaromyces	Glucose	25
Escherichia	Glucose	4.0
	Lactose	20
	Phosphate	1.6
Aspergillus	Glucose	5.0
Candida	Glycerol	4.5
	Oxygen	$0.042 \to 0.45$
Pseudomonas	Methanol	0.7
	Methane	0.4
Klebsiella	Carbon dioxide	0.4
	Magnesium	0.56
	Potassium	0.39
	Sulphate	2.7
Hansenula	Methanol	120.0
	Ribose	3.0
Cryptococcus	Thiamine	$1.4\,\mathrm{E}^{-7}$

Limit of Monod equation

The Monod equation is by far the most frequently used expression relating to growth rate to substrate concentration. However, it is valid only for balanced growth and should not be applied when growth conditions are changing rapidly. There are also other restrictions; for example, the Monod equations has been found to have limited applicability at extremely low substrate levels. When growth is inhibited by high substrate or product concentrations, extra terms can be added to the Monod equation to account for these effects.
Several other kinetic expressions has been developed for cell growth these provide better correlations for experimental data in centain situations

12.10 Production Kinetics in Cell Culture

Rate of Product Formation

$$\boldsymbol{r_P = q_P\, x} \tag{12.5}$$

$$\dim r_P = \frac{\mathrm{M/L^3}}{\mathrm{T}}; \quad \dim x = \mathrm{M\ (B)/L^3}; \quad \dim q_P = \frac{1}{\mathrm{T}}$$

r_P is the volumetric rate of production formation

x is the biomass concentration

q_P is the *specific rate of product formation*

Production rate q_P

- can be evaluated at any time during fermentation as the ratio of the production rate and biomass concentration.
- Is not necersarly constant during batch culture
- We can develop equations for q_P as a function of growth rate and other metabolic parameters (Depending on wheter the product is linked to energy metabolism or not)

12.10.1 Product Formation Directly Coupled with Energy Metabolism

For products formed using pathways that generate ATP, the rate of production is related to the cellular energy demand. Growth is usually the major energy-requiring function of cells; therefore, if production is coupled to energy metabolism, product will be formed whenever there is growth. However, ATP is also required for other activites called *maintenance*. Maintenance activities are carried out by living cells even in the absence of growth. Products synthetised in energy pathways will be produced whenever maintenance functions are carried out because ATP is required.

Examples of maintenance:

- Cell motility
- Turnover of cellular components
- Adjustment of menbrane potential and internal pH

Kinetic expressions for the rate of product formation must account for growth-associated and maintenance-associated production, as in the following equation

$$\boldsymbol{r_P = Y_{PX}\, r_X + m_P\, x} \tag{12.6}$$

$$\dim m_P = \frac{1}{\mathrm{T\,M\,(B)}}$$

r_X is the volumetric rate of biomass formation.

Y_{PX} is the theoretical or true yield of product from biomass

m_P is the *specific rate of product formation due to maintenance*

x is the biomass concentration

$$\boldsymbol{q_P = Y_{PX}\, \mu + m_P} \tag{12.7}$$

Using (12.6) , (12.5) and (12.1)

$$q_P = \frac{r_P}{x} = \frac{Y_{PX}\, r_X + m_P\, x}{x} = \frac{Y_{PX}\,(\mu\, x) + m_P\, x}{x} = Y_{PX}\, \mu + m_P$$

12.10.3 Product Formation Not Coupled with Energy Metabolism

Production not involving energy metabolism is difficult to relate to growth because growth and product synthesis are somewhat dissociated. However, in some cases, the rate of formation of nongrowth-associated products is directly proportional to biomass concentration, so that the production rate defined in (12.5) can be applied with constant q_P. Sometimes q_P is a complex function of the growth rate and must be expressed using empirical equations derived from experiment.