EB – Notebook

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Conteúdo

4 – Material Balances

4.6 Stoichiometry of Cell Growth and Production Formation

When a cell growth occurs, cells are a product of reaction and must be represented in the reaction equation. A widely used term for cells in fermentation process is textitbiomass.

In this section we discuss how reaction equations for biomass growth and product synthesis are formulated.

Metabolic stoichiometry has many applications in bioprocessing: as well as in mass and energy balances, it can be used to compare theoretical and actual product yields, check the consistency of experimental fermntation data, and formulate nutrient media.

4.6.1 Growth Stoichiometry and Elemental Balances

Despite its complexity and the thousand of intracellylar reactions involved, cell growth obeys the law of conservation of matter.

Aerobic cell growth: if only extracellular products formed are CO_2 and H_2O we can write the general equation for aerobic cell growth:

$$\mathrm{C_wH_xO_yN_z + a\,O_2 + b\,H_gO_hN_i \longrightarrow c\,CH_{\alpha}O_{\beta}N_{\delta} + d\,CO_2 + e\,H_2O}$$

Formula	For
$C_wH_xO_yN_z$	Carbon Source
$H_gO_hN_i$	Nitrogen Source
$CH_{\alpha}O_{\beta}N_{\delta}$	dry cells

Descriptio for formula

- Results are remarcably similar for different cells and conditions; $CH_{1.8}O_{0.5}N_{0.2}$ can be used as a general formula for cell biomass when composition analysis is not available.
- The average 'molecular weight' of cells based on C, H, O and N content is therefore 24.6, although $(5 \to 10)\,\%$ residual ash is often added to account for those elements not included in the formula

Balancing Aerobical cell growth

Element	Balance
C	$w = c + d$
H	$x + b\,g = c\,\alpha + 2\,e$
O	$y + 2\,a + b\,h = c\,\beta + 2\,d + e$
N	$z + b\,i = c\,\delta$

Notice that we have five unknown coefficients but only four balance equations, this means that an addition information is required before the equations can be solved. Usually this information is obtained from experiments.

A useful measurable parameter is the respiratory quotient, *RQ*:

$$\boldsymbol{RQ} = \frac{\text{mol}\,(\text{CO}_2\ \text{Produced})}{\text{mol}\,(\text{O}_2\ \text{Consumed})} = \frac{\boldsymbol{d}}{\boldsymbol{a}} \tag{4.1}$$

limit of chemical balance Although elemental balances are usefull, the presence of water cause some problems in pratical application. Becausewater is usually present in great excess and changes in water concentration are incovenient to measure or experimentally verify, H and O balances can present difficulties.

Example 4.7

Production of single-cell protein ffrom hexadecane is descibed by the following equation:

$$C_{16}H_{34} + a\,O_2 + b\,NH_3 \longrightarrow$$
$$c\,CH_{1.66}O_{0.27}N_{0.20} + d\,CO_2 + e\,H_2O$$

Where $CH_{1.66}O_{0.77}N_{0.20}$ represents the biomass. if $RQ = 0.43$, determine the scoichiometric coefficients.

Resposta

Balancing

$$\begin{cases} C: & 16 = c + d \\ H: & 34 + b\,3 = c\,1.66 + 2\,e \\ O: & 2\,a = c\,0.27 + 2\,d + e \\ N: & b = c\,0.20 \\ RQ: & 0.43 = d/a \end{cases}$$

$$\left\{\begin{array}{ccc} c & = & 16 - d \\ a & = & d/0.43 \\ b & = & (16-d)\,0.20 \\ e & = & \frac{1}{2}(34 + 3\,((16-d)\,0.20) - (16-d)\,1.66) \\ 2\,a & = & c\,0.27 + 2\,d + e \end{array}\right\} = \left\{\begin{array}{cc} c & \cong 10.630 \\ a & \cong 12.488 \\ b & \cong 53.151 \\ e & \cong 11.366 \\ d & \cong 05.370 \end{array}\right\}$$

$d:$

$$2\,a = 2\,(d/0.43) =$$
$$= c\,0.27 + 2\,d + e =$$
$$= (16-d)\,0.27 + 2\,d + \frac{1}{2}(34 + 3\,((16-d)\,0.20) - (16-d)\,1.66) \implies$$
$$\implies d = \frac{16 * 0.27 + 17 + \frac{1}{2}(3 * 16 * 0.20 - 16 * 1.66)}{\frac{2}{0.43} + 0.27 - 2 + (3 * 0.20 - 1.66)/2} \cong 5.370$$

4.6.2 Electron Balance

A usefull principle is conservation of reducting power or available electrons, which can be applied to determine quantitative relationships between substrates and products. **An electron balance shows how available electrons from the substrate are distributed during reaction**.

Available electrons (ae) refers to the number of electrons availabe for transfer to oxygen on combustion of a substance to CO_2, H_2O and nitrogen-containing compounds.

The number of available electrons is calculated from the valence of the various elements of the molecule.

Molecule	Element	valence or reference state
Ammonia	N	-3
Nitrogen (molec)	N_2	0
Nitrate	N	5
Organics	C	4
	H	1
	O	-2
	P	5
	S	6

Degree of reduction γ is defined as the number of equivalents of available electrons that quantity of material containing $1\,\mathrm{g}_{\text{Atom of C}}$.

For the substrate ($C_wH_xO_yN_z$):

$$\gamma_S = \frac{ae_S}{w} = \frac{4\,w + x - 2\,y - 3\,z}{w} \tag{4.2}$$

Note that the number of available electrons and the degree of reduction of CO_2, H_2O, and NH_3 are zero, This means that the stoichiometric coefficients for these componds do not appear in the electron balance, thus simplifying balance calculations.

Available electrons are conserved during metabolism. In a balanced growth equation, the number of available electrons is conserved by virtue of the fact that the amounts of each chemical element are coserved.

Available electron balance considering

- Conservation of available electrons during metabolism
- With ammonia as the nitrogen source
- $ae_{CO_2} = ae_{H_2O} = ae_{NH_3} = 0$
- $S = C_w\,H_x\,O_y\,N_z$
- $B = CH_\alpha O\beta N\delta$

$$S + a\,O_2 + b\,NH_3 \longrightarrow c\,B + d\,CO_2 + e\,H_2O$$

$$w\,\gamma_{Substrate} + a\,(-2 * 2) = w\,\gamma_{Substrate} - 4\,a = c\,\gamma_{Biomass} \tag{4.3}$$

4.6.3 Biomass Yield

Typically, the equation for electron balance is used with carbon and nitrogen balances, elemental balances and the value of respiratory quotient, *RQ*, for evaluation os stoichiometric coefficients. However, as those are inadequate information for solution of five unkown coefficients, another experimental quantity is required.

During cell growth there is, as a general approximation, a **linear relationship between the amount of biomass produced and the amount of substrate consumed**. This relationship is expressed quantittively using the **biomass yield, Y_{XS}**

$$\boldsymbol{Y_{XS}} = \frac{\text{g (Cells produced)}}{\text{g (Substrate consumed)}} \tag{4.4}$$

A large number of factors influence biomass yield, including:

- Medium composition
- pH and Temperature
- $Y_{XS,\text{Aerobic culture}} > Y_{XS,\text{Anaerobic culture}}$
- Nature of the carbon source
- Nature of the nitrogen source
- Choice of Electron receptor (eg: O_2, nitrate, or sulphate)

Elemental balance using Yield

When Y_{XS} is constant throught growth, its experimentally determined value can be used to evaluate the stoichiometric coeficient for biomass produced (c)

$$\boldsymbol{Y_{XS}} = \frac{\boldsymbol{c * (MW_{\text{Cells}} + r)}}{\boldsymbol{MW_{\text{Substrate}}}} \tag{4.5}$$

MW stands for Molecular Weight; r stands for residual ash

Limits: We must be sure that the substrate is fully used to produce biomass. One complication with real cultures is that some fraction of substrate consumed is always used for maintenance activities. These metabolic functions require subtrate but do not necessarily produce cell biomass, CO_2, and H_2O in the way described by the element balance. (check Chapter 12 for further discussion)

4.6.4 Product Stoichiometry

In many fermentations, extracellular products are formed during growth in addition to biomass. When this occurs, the stoichiometric equation can be modified to reflect product synthesis.

Consider the formation of an extracellular product $C_jH_kO_lN_m$, the elemental balance can be extended to include the product:

$$C_wH_xO_yN_z + a\,O_2 + b\,H_gO_hN_i \longrightarrow$$
$$c\,CH_\alpha O_\beta N_\delta + d\,CO_2 + e\,H_2O + f\,C_jH_kO_lN_m$$

Product synthesis introduces one extra unknown stoichiometric coefficient to the equation; thus an **additional relationship between the determinants and products is required**. This is usually provided as another **experimentally determined** yield coefficient, **the product yield from substrate, Y_{PS}**

$$\boldsymbol{Y_{PS}} = \frac{\text{g (Product formed)}}{\text{g (Substract consumed)}} = \frac{\boldsymbol{f\,MW_{P\text{roduct}}}}{\boldsymbol{MW_{S\text{ubstract}}}} \tag{4.6}$$

Limits: **this does not hold if product formation is not directly linked to growth**; thus **it can not be applied for secondary metabolite production** such as penicillin fermentation, or for biotransformations such as steroid hydroxylation tha involve only a small number of enzymes in cells. In these cases independent reaction equations must be used to describe growth and product synthesys

4.6.5 Theorical Oxygen Demand

As oxygen is often the limiting substrate in aerobic fermentations, oxygen demand is an important parameter in bioprocessing. Oxygen demand is represented by the stoichiometric coefficient a in the balance equations.

The required oxygen demand is related directly to the electrons available for transfer to oxygen; deriving from appropriate electron balance:

- S: $C_wH_xO_yN_z$
- B: $CH_\alpha O_\beta N_\gamma$
- P: $C_jH_kO_lN_m$

$$S + a\,O_2 + b\,NH_3 \longrightarrow c\,B + d\,CO_2 + e\,H_2O + f\,P$$

$$a = \frac{1}{4}(w\,\gamma_S - c\,\gamma_B - f\,j\,\gamma_P) \tag{4.7}$$

$$\Longleftarrow\ w\,\gamma_S + a\,\gamma_{O_2} = w\,\gamma_S - 4\,a = c\,\gamma_B + f\,j\,\gamma_P$$

Note: this is easier to calculate as is does not require that the quantities for NH_3, CO_2 and H_2O involved in the reaction to be known.

4.6.6 Maximum Possible Yield

Consider de the fractional allocation of available electrons in the substrate for defining $c_{\max}$ **and** $f_{\max}$

$$1 = \frac{4\,a}{w\,\gamma_S} + \frac{c\,\gamma_B}{w\,\gamma_S} + \frac{f\,j\,\gamma_P}{w\,\gamma_S} \tag{4.8}$$

Let us define ζ_B as the fraction of available electrons in the substrate transfered to biomass

$$\zeta_B = \frac{c\,\gamma_B}{w\,\gamma_S} \tag{4.9}$$

In the abscense of product formation, if all vailable electrons were used for biomass synthesis, ζ_B would equal unity.

Defining $c_{\max}$

Under these conditions, the maximum value of the stoichiometric coefficient c is:

$$c_{\max} = w\,\gamma_S/\gamma_B \tag{4.10}$$

- The $c_{\max}$ can be converted to biomass yield with mass units using $Y_{XS} = \frac{c\,(MW_{\text{Cells}}+r)}{MW_{\text{Substrate}}}$. Therefore if we do not know the stoichiometry of growth, we can quickly **calculate an upper limit for biomass yield** from the molecular formulae for the substrate and product
- If the compostion of the cells is unknown, γ_B can be taken as 4.2 corresponding to the average biomass formula ($CH_{1.8}O_{0.5}N_{0.2}$)
- The maximum biomass yield can be expressed in therms of mass ($Y_{XS,\max}$), or as the number of C atoms in the biomass per substrate C-atom consumed ($c_{\max}/w$). These quantities are sometimes know an *thermodynamic maximum biomass yields*.
- Substrates with high energy content, indicated by high Y_S values, give high maximum biomass yields.

Maximum possible product yield $f_{\max}$

In the abscensse of biomass synthesis can be determined

$$f_{\max} = w\,\gamma_S/j\,\gamma_P \tag{4.11}$$

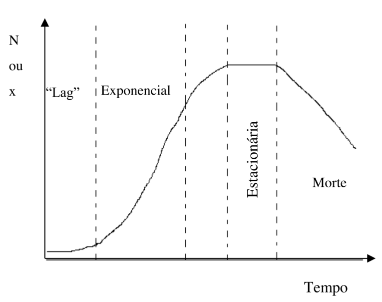
Allows us to quickly calculate an upper limit for the product yield from the molecular formulae for the substrate and product

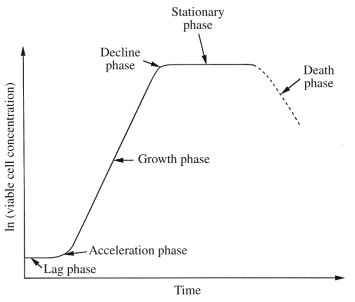
12 – Homogeneous Reactions

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

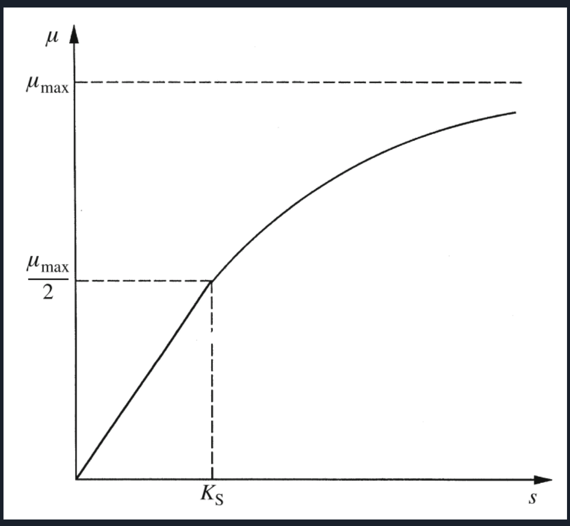
During the growth and decline phases of batch culture, the specific growth rate of the cells depends on the concentration of nutrientes in the medium.

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:

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

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



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.