Modeling cellular processes
Definitions

1 Balance spaces

Describing concentration behavior by setting up balances requires the definition of the measure for an amount and the balance space. For the description of cellular processes, there are major spaces to take into account in the bioreactor:

- Extracellular liquid space (filtrate – without the space taken by cells)
- Gas-phase (all bubbles and headspace)
- Intracellular (metabolite concentrations, protein content, C-content, ..)

Note:

Bioreactor = liquid + biomass + gas

Broth = liquid + biomass

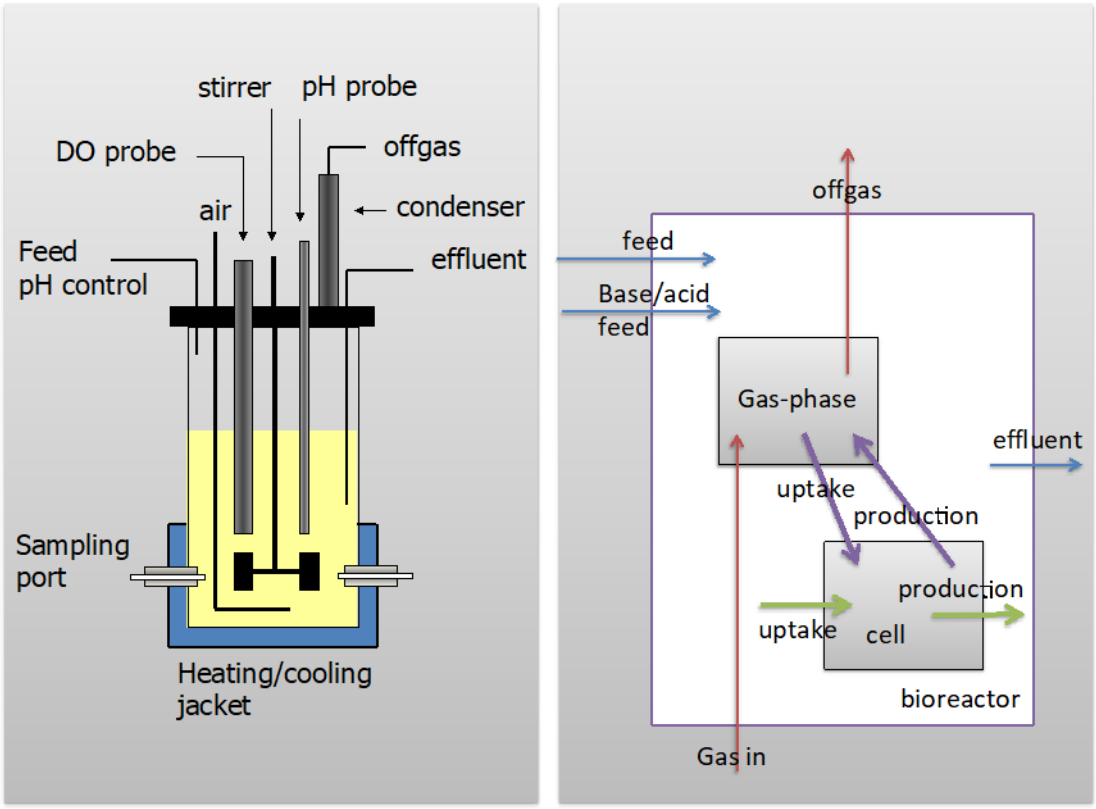


Figure 1, left: Bioreactor setup, right: balance spaces used for modeling cellular processes. The bioreactor has exchange fluxes with the 'outside' by the feed, base/acid feed and effluent (blue). Cells exchange compounds with the liquid phase (bioreactor in green) and the gaseous phase (in red). In section 1 the definitions for quantification of compounds in the single phases will be discussed, in section 2 the balances and connections of the different spaces will be described.

1.1 Broth

For the broth, the content in the liquid phase of the bioreactor is balanced. This includes:

- Biomass (c_X with the units g_{DM}, C-mol, L_{ic}, …)
- Extracellular metabolites (substrate, product, by-products, N-source, … mostly measured in mol, g or C-mol)

The balance space bioreactor can have different measures:

- Volume (L): describes the volume of liquid in the bioreactor.
  Broth: 'total' liquid volume, e.g. water and all components including biomass. We will use the notation V_L
  Filtrate: This is the volume without (wet) biomass: Use the notation V_F
- Mass (kg): describes the mass of broth or filtrate. These are denoted with M_L and M_F respectively.
  (thus, the **wet** volume and mass of biomass is then: $V_X = V_L - V_F$ and $M_X = M_L - M_F$)

For low biomass concentrations, broth and filtrate are very comparable while in high-cell-density cultivations, significant differences can occur (e.g. 100 g_{DM}/L_{broth} – here the wet biomass takes a significant amount of volume ~20%).

Note: There is no volume balance – the volume can change depending on the composition of the liquid (for example EtOH/water mixtures). Thus, the balance is always a mass-balance.

1.2 Quantification of biomass

Biomass can be described in different ways:

1. Counts
2. Mass (wet and dry, with or without ash)
3. C-mol

Depending on how well the biomass is characterized, conversion factors for the different measures are available (see table 1)

1.2.1 Cell numbers

Especially in mammalian cell culturing, biomass is quantified by the number of cells. Different devices are available that are able to count cells, e.g. using a microscope, plates (colony forming units), Coulter counter, Flow cytometry (FC), and indirect methods like capacity which require calibration. The biomass concentration (as number/volume) is mostly determined with respect to the broth volume (e.g. the sample is representative for the broth):

$$c_X = \frac{n_{\text{sample}}}{V_{\text{sample}}} \tag{1}$$

Cell numbers can be of advantage to quantify different types of cells (e.g. in specific state of the cell cycle) using FC with dyes.

Also for mixed cultures, cell numbers can be obtained using molecular methods e.g. qPCR (real time PCR) of cell specific DNA. Note, that with this method, artifacts can occur (bacteria can contain up to eight copies of DNA).

1.2.2 Cell mass

The most common unit used in biotechnology is cell dry weight. This measure is based on weighing cells that were dried, e.g. contain very low amounts of extracellular and intracellular water. Usually, a defined amount of broth is filtered, washed and dried (e.g. 70 ˚C) to a steady-state mass. The weight difference of the dried filter and the filter containing the dried biomass gives the concentration in the bioreactor:

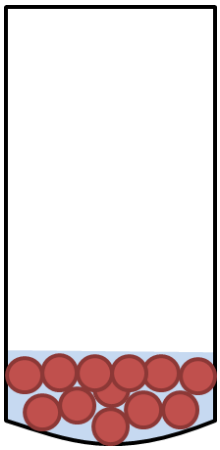
$$c_X = \frac{m_{\text{filter+bm}} - m_{\text{filter}}}{m_{\text{sample}}} \tag{2}$$

Note, that m_{sample} is the sample weight, resulting in a concentration of biomass with respect to broth weight.

If the sample amount is determined volumetricly, e.g. by using a pipette, m_{sample} will be replaced by V_{sample}.

To indicate the measurement of dry biomass, the unit is extended with an index DM (dry mass) e.g. g_{DM}

Alternatively, the wet biomass amounts can be measured with comparable methods (mass of wet loaded filter – mass of wet filter). To indicate this measure, the index WM (wet mass) resp. biomass CM (cake mass) is used.



Another method is to determine wet biomass is to use the pellet mass (PM). E.g. the sample is centrifuged instead of filtration. As shown in Fig. 2, the pellet contains biomass (with intracellular water) and intercellular water (between the cells).

Note: Wet biomass determination is a 'risky' measure. Depending on the cell shape and the filtration/centrifugation conditions, the amount of water between the cells can vary. Therefore dry mass is the preferred measure.

Summarizing, to quantify the mass of biomass we can use $\text{g}_{\text{DM}}, \text{g}_{\text{WM}}, \text{g}_{\text{CM}}, \text{g}_{\text{PM}}$.

1.2.3 Cell components

In some cases, a specific component of the biomass is measured:

- Protein weight using assays like Bradford or BCA.
- Amount of carbon using TOC measurements
- Amount of nitrogen using Kjelldahl assay
- COD: chemical oxygen demand (e.g. in waste-water treatment)
- ODM: organic dry matter (dry biomass is burned and the inorganic remainders (ash) are substracted)

1.2.4 Choice of measure

The choice of measure depends on

- the available measurement technologies
- the purpose of the modeling approach (very important)

E.g. for black-box approaches, the most convenient unit is C-mol$_X$. This unit simplifies stoichiometric calculations. Additionally, the elemental composition of biomass can be measured to also close the balances for the other elements (N, O, P, H).

In general, the different biomass units can be converted into each other. There are two main numbers required: (1) water content and (2) biomass composition.

General number are 60-70% intracellular water content – this can e.g. be determined from dry vs. wet measurements.

The biomass composition is condition dependent, e.g. the protein content is between 10%-90% (for yeast ~40%), RNA 5%-8%, DNA ~1%.

1.3 Gas-phase

For the gas-phase, care has to be taken on several parameters: Temperature, Pressure, water content, etc.

Usually, a flow rate is determined using a mass flow controller (based on the inflow). The flow rate displayed reflects the amount (mol) of gas at standard conditions (T=25C, p=1 bar).

In case air is used, a standard composition of 78.08% N_2 and 20.95% O_2 and 0.03% CO^2 is assumed (the rest are e.g. argon, helium, …). Note that dry air should be used.

Measurements in the off-gas are performed on 'dried' air, and the result is a volume percentage (equivalent to molar fraction) of a certain component (CO_2, O_2) in the off-gas flow, e.g. $mol_{CO2}/mol_{gas,out}$. Note, that in most cases, the remaining component (unmeasured) is N_2. Clearly, if the bio-process has a volatile product (e.g. methane) these products also have to be taken into account in the gas-phase balancing.

Note: The gas outflow rate is always calculated using the N_2 balance (except $N_{2(g)}$ producing bacteria cultivations).

1.4 Intracellular space

The intracellular space boundary is the cell-wall[1]. There are several components that can be balanced in the intracellular space, here we focus on three classes: **metabolites**, **enzymes** (including transporters) and **mRNA**.

Metabolite concentration: Intracellular metabolites are measured with sensitive LC- or GC-MS devices. The signal (ion-counts) is translated via a calibration line to mol/L_{sample}. Based on the sampling processing steps, the concentration in the sample can be converted to metabolite amount (mol) per amount of cells (measured as numbers, weight, volume). The amount of metabolite per amount of cell is defined with the letter x and the metabolite name as index. In most cases, the metabolite amount per cell is quantified using:

$$\frac{\mu\mathrm{mol}}{\mathrm{g_{DM}}}, \frac{\mu\mathrm{mol}}{\mathrm{Cmol}_X}, \frac{\mu\mathrm{mol}}{\mathrm{cell}}$$

Especially for calculations including thermodynamics, transport processes and enzyme kinetics, we **need** to define the intracellular metabolite concentration in

$$x_{\text{metabolite i}}\left[\frac{\mathrm{mol}_i}{\mathrm{L_{ic}}}\right] \tag{3}$$

With a proper characterization of the biomass, the unit can be converted (see table 1).

For proper kinetic formulation, also the concentration of the enzyme is required. The **quantification of enzyme** is challenging (see next paragraph). For a moment, we assume that the amounts can be determined. Enzyme can be quantified using several units: (1) mol, (2) g, (3) C-mol, (4) activity. For the enzyme concentration, the letter e_i with index i identifying the enzyme (e.g. HXK for Hexokinase). Because metabolites are defined with respect to the cellular volume, also e will be defined with respect to the intracellular volume:

$$e_i\left[\frac{\mathrm{mol}}{\mathrm{L_{ic}}}\right] \tag{4}$$

As mentioned, in most cases, the concentration cannot be measured. Most relevant is the flux that can be obtained with the enzyme amount present in the cell *in-vivo*. In general, the flux of an enzymatic reaction depends on the enzyme concentration (e_i), the specific catalytic activity $k_{cat,i}$ and the metabolite concentrations x (substrate, product, effector). The specific catalytic activity describes how much product can be produced per amount of enzyme (at saturation: all required substrates are provided in excess, such that the maximal conversion rate is obtained). As an example, we take the hexokinase reaction:

[1] Eukaryotic cells do have different cellular compartments, which result in different balance spaces. Here we ignore compartmentation and always take a 'homogenized' cytosolic space into account.

$$\mathrm{Glc} + \mathrm{ATP} \xrightarrow{HXK} \mathrm{G6P} + \mathrm{ADP} \tag{5}$$

Per definition, the enzyme specific rate of an enzymatic reaction is determined as product produced per time and amount of enzyme. In enzymology, the most common unit is U:

$$k_{cat,i} = \frac{N_{\mathrm{product}} \ / \ t}{N_i} \left[\mathrm{U} = \frac{\mu\mathrm{mol/min}}{\mathrm{mg_i}} \right] \tag{6}$$

In most cases, the specific activity $k_{cat,i}$ is not available. Therefore, a simplification is made, e.g. replacing N_i by an amount that can be measured. Most commonly, the amount of total protein in the cell extract (measured via BCA assay) – this value is defined as *in-vitro* maximal enzyme capacity: $v_{\mathrm{max},i}$:

$$v_{\mathrm{max},i} = \frac{N_{\mathrm{product}} \ / \ t}{M_{\mathrm{protein}}} \left[\mathrm{U} = \frac{\mu\mathrm{mol/min}}{\mathrm{mg_{protein}}} \right] \tag{7}$$

A special case are membrane proteins, especially transporters. Here the concentration with respect to the cell surface is the relevant description, determining the amount of available transporters per cell.

Next to the metabolite and enzyme levels, the concentration of mRNA is relevant. The concentration of mRNA is assumed to determine the production rate of enzymes (several other mechanisms are involved, mRNA is not the only influencing factor). In most cases, mRNA levels are measured relative to a reference mRNA sample. As a result, the mRNA expression level is expressed as X fold up- or downregulated.

For the purpose of modeling, mRNA is quantified as copy numbers which can be determined using RT-qPCR (retro transcriptase - quantitative PCR).

2 Balances

The definitions of section 1 have an influence on the balance formulation, especially in which units these are defined. The balances are always derived from the general mass balance, e.g. the accumulation/depletion of a component i inside the balance space depends on:

$$accumulation = transport - conversion \tag{8}$$

In the following, the balances for different system boundaries are described.

2.1 Black-box (bioreactor models)

The classical black box model only contains two balance spaces, the broth and the gas-phase.

Example:

The biomass concentration

c_X is defined as amount of cells/volume (counts / L_{broth}).

The substrate (glucose)

c_S is described in amount/volume (mol/L_{broth})

The excreted product (enzyme)

c_P is described in activity / volume (U/L_{broth})

CO_2 in the offgas

$y_{CO2,out}$ or y_C measured in the gas as molfraction – which mostly is expressed in %.

The electron acceptor

$y_{O2,in}$ (O_2) in the inflow is 20.95% (air) and is measured in the offgas in $y_{O2,out}$ (%)

The ingas flow $F_{n,in}$ is determined in mol / h (mass flow controller)

The liquid phase (broth) in the bioreactor is V_L in L_{broth}.

The process is conducted in fed-batch mode in a 20 L bioreactor (starting broth volume $V_L(0) = 5\,\mathrm{L}$) with a feed $F_{in}\,(\mathrm{kg/h\,or\,mol/h})$ containing fresh medium with substrate (concentration $c_{\mathrm{S,in}}\left[\frac{\mathrm{mol_S}}{\mathrm{L_{feed}}}\right] or \left[\frac{\mathrm{mol_S}}{\mathrm{kg_{feed}}}\right]$).

As a consequence of the above mentioned definitions, the balances in amounts/h are now formulated as (note, these all have their own unit):

Biomass:	cells / h
Substrate:	mol_S / h
Product (enzyme):	U_P / h
Product (CO_2):	mol / h
e^- acceptor (O_2):	mol / h

In special, following balances are used:

Volume – note, this only holds true for diluted systems (not for high cell density or feeds with very high substrate concentrations)

$$\frac{dV_L}{dt} = F_{in} \quad \text{(see remark in text)} \tag{9}$$

Biomass:

$$\frac{d\ V_L\, c_X}{dt} = \mu\, V_L\, c_X \tag{10}$$

Note, that growth in this case has a unit $\left[\frac{\text{number}_{\text{cells produced}}\ /\ h}{\text{number}_{\text{cells present in the reactor}}}\right]$

Cellular growth can also be in size and mass only, without cell division. Using different measures for cell amounts (mass, numbers, volume) growth has different interpretations.

Substrate:

$$\frac{d\ V_L c_S}{dt} = F_{in}\, c_S - q_S V_L c_X \tag{11}$$

The uptake rate q_S is defined in $\left[\frac{\text{mol}_S\ /\ \text{h}}{\text{cell}}\right]$

Note: The uptake can be defined in different directions. Assuming the flux as inflow into the cell, q_S is a sink: $-q_S V_L c_X$. q_S is positive when the cells consume substrate.

If defined as transport into the broth, it will appear as $+q_S V_L c_X$ in the balance. q_S is negative when cells consume substrate.

Product:

$$\frac{d\ V_L c_P}{dt} = q_P V_L c_X \tag{12}$$

The enzyme production rate is defined in $\frac{\text{U/h}}{\text{cell}}$

Gas-phase – O_2 and CO_2

Strictly, the organism has no direct interaction with the gas-phase. The transfer of O_2 and CO_2 is via the liquid phase. The transport between the liquid and gas-phase is described by the transfer rate T_{O2} and T_{CO2}. We can now write:

$$\begin{aligned} \frac{dN_G\, y_{O2}}{dt} &= F_{n,in}\, y_{O2,in} - F_{n,out}\, y_{O2,out} - T_{n,O2} \\ \frac{dV_L\, c_{O2}}{dt} &= F_{in,O2}\, c_{O2,in} - F_{out,O2}\, c_{L,O2} - q_{O2}\, c_X\, V_L + T_{n,O2} \end{aligned} \tag{13}$$

Simplifications:

The content of dissolved oxygen (resp. dissolved CO_2) in the feed and the liquid phase is very small ($c_{O2,in} = 0, c_{O2} = 0$). This immediately also leads to $\frac{dV_L\, c_{O2}}{dt}$ being neglected, thus $\frac{dV_L\, c_{O2}}{dt} \approx 0$. The gas-phase amount N_G present in the bioreactor is usually relatively small compared to the applied flow rate. Assuming pseudo-steady state, the accumulation term of the gas balances can be neglected: $\frac{dN_G\, y_{O2}}{dt} \approx 0$. Together with the simplified balances we obtain:

$$\begin{aligned} 0 &= F_{n,in}\, y_{O2,in} - F_{n,out}\, y_{O2,out} - T_{n,O2} \quad (\text{gas O}_2) \\ 0 &= 0 - 0 - q_{O2}\, c_X\, V_L + T_{n,O2} \quad (\text{broth O}_2) \\ \hline 0 &= F_{n,in}\, y_{O2,in} - F_{n,out}\, y_{O2,out} - q_{O2}\, c_X\, V_L \end{aligned} \qquad (14)$$

Because of the biomass production of CO_2 and consumption of O_2, the molar gasflow of the in-and outflow are not equal. Fortunately, it can be assumed, that N_2 is the only additional component in air and offgas (if no volatile products are formed). N_2 is not participating in any reactions. Thus, the gas in and out can be formulated as:

$$\begin{aligned} F_{G,out}\, y_{N2,out} &= F_{G,in}\, y_{N2,in} \\ y_{N2,out} + y_{O2,out} + y_{CO2,out} &= 1 \end{aligned} \qquad (15)$$

2.2 Intracellular balances

For the intracellular space, the metabolite balance applies (8). Special attention has to be laid on transport processes, the so called extracellular fluxes (e.g. fluxes crossing the membrane). Here substrate and product are not in the same balance space. For example, the substrate uptake transports glucose from the extracellular space over the membrane into the intracellular space. The balance for extracellular substrate applies to the broth balance space. The balance for intracellular glucose into the cellular balance space.

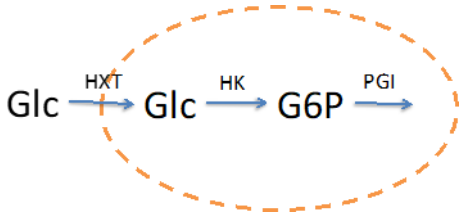


Figure 2: Small example network with the uptake reaction HXT and two intracellular fluxes HK and GPI. (outside: broth balance space, inside: intracellular space)

Metabolite balances

Metabolites are only balanced in the intracellular space, e.g. extracellular Glc in Figure 2 will not occur in the balances. Nevertheless, the transport flux mediated by HXT will appear, as it has an influence on the intracellular Glc pool. As for the extracellular space, we first define the measures of metabolites.

As an example, we define that metabolites are quantified with respect to the intracellular volume, resulting in a molar intracellular concentration.

$$x_{Glc}, x_{G6P} \quad \left[\frac{\text{mol}}{\text{L}_{\text{ic}}}\right] \qquad (16)$$

The metabolite balance now takes the whole intracellular space into account, e.g. the **cellular volume present in the whole bioreactor**.

For intracellular Glc[2] the balance reads:

$$\frac{d\ V_{ic}\, x_{Glc}}{dt} = v_{HXT} V_{ic} - v_{HK}\, V_{ic} \tag{17}$$

The (overall) intracellular volume can usually not be measured directly. For the running example, we assume that the biomass is well characterized, and the specific volume of the cells is known

$$\nu = 2 \cdot 10^{-18}\, \frac{\mathrm{L_{ic}}}{\mathrm{cell}} \tag{18}$$

Knowing this relation now allows to determine the total intracellular volume in the bioreactor:

$$V_X = \nu\, c_X V_L \tag{19}$$

Eq. (17) can then be rewritten as:

$$\frac{d\ \nu\, c_X V_L\, x_{Glc}}{dt} = v_{HXT}\, \nu\, c_X V_L - v_{HXK}\, \nu\, c_X V_L \tag{20}$$

Note, that v_{HXT} and q_S both describe the rate of the glucose transport mechanism, but in different units: q_S is measured per cell number, while the intracellular flux v_{HXT} is in reference to the intracellular volume. With the given relation between cell number and intracellular volume, v_{HXT} can be derived from q_S: (additional note: check the flux directions of q_S and v_{HXT}, in the classical definition, q_S is defined as export, e.g. $-q_S$ is uptake)

$$v_{HXT} = -\frac{q_S}{\nu} \tag{21}$$

In a detailed kinetic model v_{HXT} will be a function of the extra- and intracellular glucose concentrations (if assumed reversible, see transport mechanisms). Note the differences in the balance equations for intra-and extracellular substrate:

$$\begin{aligned} \frac{d\ c_S\, V_L}{dt} &= F_{in}\, c_{S,in} - q_S\, c_X\, V_L - F_{out}\, c_S\, V_L \\ \frac{d\ x_{Glc}\, \nu\, c_X\, V_L}{dt} &= +v_{HXT}\, \nu\, c_X\, V_L - v_{HXK}\, \nu\, c_X\, V_L \end{aligned} \tag{22}$$

On the left side of the differential equation, we find besides the concentration and volume also the biomass concentration c_X. c_X is found in the broth biomass balance and depends on the growth rate and inflows to the bioreactor, thus the derivative is **not zero**. With a set of assumptions (e.g. ν is constant) and combining with the biomass balance in Eq. (10), we can replace $\frac{d\ \ c_X\, V_L}{dt} = \mu\, c_X\, V_L$ and eq. (22) results in:

$$\begin{aligned} &\frac{d\ x_{Glc}\, \nu\, c_X\, V_L}{dt} = -v_{HXT}\, \nu\, c_X\, V_L - v_{HXK}\, \nu\, c_X\, V_L \\ &\frac{d\, x_{Glc}}{dt}\ \nu\, c_X\, V_L\ + x_{Glc} \frac{d\ \ \nu\, c_X\, V_L}{dt} = -v_{HXT}\, \nu\, c_X\, V_L - v_{HXK}\, \nu\, c_X\, V_L \\ &\frac{d\, x_{Glc}}{dt} + x_{Glc}\, \mu = +v_{HXT} - v_{HXK} \\ &\frac{d\, x_{Glc}}{dt} = +v_{HXT} - v_{HXK} - x_{Glc}\, \mu \end{aligned} \tag{23}$$

In most cases, the metabolic fluxes (v_{HXT}, v_{HXK}) will be much larger than the contribution of $x_{glc}\, \mu$ (the so called dilution term) and Eq. (23) further simplifies to:

[2] we assume that glucose is not degraded or produced from other sources.

$$\frac{d\,x_{Glc}}{dt} = +v_{HXT} - v_{HXK} \tag{24}$$

Note, that this assumption is only valid for low concentrated metabolites with fast turnover (e.g. G6P has a turnover time of about 0.5 s, the time of growth is in the order of hours). For storage metabolites like glycogen or others, the turnover flux is much lower and the concentration much higher and the dilution term cannot be neglected.

Further contributions to the metabolite balances:

The Biomass synthesis effluxes

Most models will include reactions that form biomass from balanced key metabolites. The flux of key metabolites towards the biomass are derived from the biomass composition, e.g. 60% protein, 7% RNA, 1% DNA, 20% Lipids, 12% Carbohydrates. The percentages relate to weight/weight. Furthermore, the composition (amino-acids) of protein is known, e.g. for alanine 11% ($g_{Ala}/g_{Protein}$). With this data the amount of Alanine to produce 1g of biomass can be calculated $d_{ala} = 0.6\frac{\mathrm{g_{Protein}}}{\mathrm{g_{DM}}} \cdot 0.11\frac{\mathrm{g_{Ala}}}{\mathrm{g_{Protein}}} = 0.066\frac{\mathrm{g_{ala}}}{\mathrm{g_{DM}}}$. At a given growth rate the amount of $v_{ala,BM} = \mu\, d_{ala}$ is required. Thus, a series of conversions is required to obtain instead of $\frac{\mathrm{g_{ala}/h}}{\mathrm{g_{DM}}}$ the unit of an intracellular flux $\frac{\mathrm{mol_{ala}/h}}{\mathrm{cell}}$ towards biomass formation.

Here, we assume, that 1 cell has an average wet weight of 2.5 ng. Usually 70% of the cell weight is water, thus a dry weight of 0.75 ng is calculated. Thus, 1 g_{DM} contains 1.33 E9 cells. The molar weigth of alanine (minus water) is 71 g/mol. With these numbers at hand we can now calculate the requirements for growth in $mol_{ala}/L_{ic}/h$.

Enzyme balances, kinetics

In principle, mRNA and enzymes are ‘large metabolites’ that are synthesized and degraded like metabolites. But, in practice the measurement technologies are not yet available to quantify the amounts. Therefore, simplifications are introduced on several levels. E.g. the degradation is assumed to be comparable for all enzymes, the concentrations are replaced by activities which can be measured much more easily. The level of mRNA is mostly ignored, e.g. a signal directly translates into protein production rates.

On the side of kinetics, mostly Michaelis-Menten type of kinetics are applied. Note, that the v_{max} value is a combination of the enzyme amount (in enzymology E0) and the specific activity (mass action term k of the last step for a simple irreversible reaction).

Thus, models containing balanced enzymes usually balance activity e_i (U):

$$\frac{d\;\; e_{HXK}\,\nu\,c_X\,V_L}{dt} = +v_{e,HXK}\,\nu\,c_X\,V_L - k\;e_{HXK}\,\nu\,c_X\,V_L \tag{25}$$

The kinetic function for a flux like v_{HXK} then can be formulated as:

$$v_{HXK} = e_{HXK}(t)\,f(\mathbf{x}) \tag{26}$$

With f(x) being a non-linear function of the involved metabolite concentrations (substrates, products, effectors).

3 Transport processes

Diffusion depends on mostly three parameters: (1) The permeability $\kappa_i \left[\frac{\mathrm{m}}{\mathrm{s}}\right]$ of the membrane for component i, (2) the cell surface $A \left[\mathrm{m}^2\right]$ and (3) the thermodynamic driving force resp. 'distance' to the equilibrium concentrations (v_i defined from ic to liquid phase):

$$v_i = \kappa_i \frac{A}{V_{ic}} \left(x_{i,ic} - \frac{c_{i,L}}{K_{eq}} \right) \tag{27}$$

In most cases, diffusion applies for uncharged molecules which have a K_{eq} = 1 (in/outside). Values different from 1 could occure in cases where the influence of different ionic strength plays a role.

Note, that A now refers to the membrane surface with respect to the intracellular volume (L_{ic}). For the running example, the value reported is $1600 \frac{\mu\mathrm{m}^2}{\mathrm{cell}}$, with the known volume $\nu = 2.5 \cdot 10^{-12} \frac{\mathrm{L_{ic}}}{\mathrm{cell}}$ we obtain a specific surface of $\frac{A}{V_{ic}} = 640 \frac{\mathrm{m}^2}{\mathrm{L_{ic}}}$. Typical values of κ are in the range of $\kappa \sim 5 \cdot 10^{-7} \frac{\mathrm{m}}{\mathrm{s}}$.

Note, that the rate is now a specific rate $v_i \left[\frac{\mathrm{mol}_i}{\mathrm{L_{ic}\,s}}\right]$ with respect to the intracellular volume. To convert it to a 'black-box' rate e.g. $q_i \left[\frac{\mathrm{mol}_i}{\mathrm{cell\,s}}\right]$ use the specific volume:

$$q_i = \frac{v_i}{\nu} \tag{28}$$

Active transport processes are described in detail in the respective syllabus.

Tabel 1: Typical characteristics of cells

	Cell volume	Cell weightl	Cell surface	Protein content	RNA content	Water
HeLa	$\nu = 2.5 \cdot 10^{-12} \frac{\mathrm{L_{ic}}}{\mathrm{cell}}$	$2.5 \frac{\mathrm{ng_{WM}}}{\mathrm{cell}}$ $0.75 \frac{\mathrm{ng_{DM}}}{\mathrm{cell}}$	$1600 \frac{\mu\mathrm{m}^2}{\mathrm{cell}}$	15-65 % (w/w)	5-10 % (w/w)	
Yeast	$\nu = 25...75 \cdot 10^{-15} \frac{\mathrm{L_{ic}}}{\mathrm{cell}}$ $\bar{\nu} = 42 \cdot 10^{-15} \frac{\mathrm{L_{ic}}}{\mathrm{cell}}$	$0.025...0.075 \frac{\mathrm{ng_{WM}}}{\mathrm{cell}}$	$60 \cdot 10^{-12} \frac{\mathrm{m}^2}{\mathrm{cell}}$	~40%	~7%	68%

Jerry W. King, Gary R, Supercritical fluid technology in oil and lipid chemistry, 1996, American Oil Chemists' Society, pp. 303