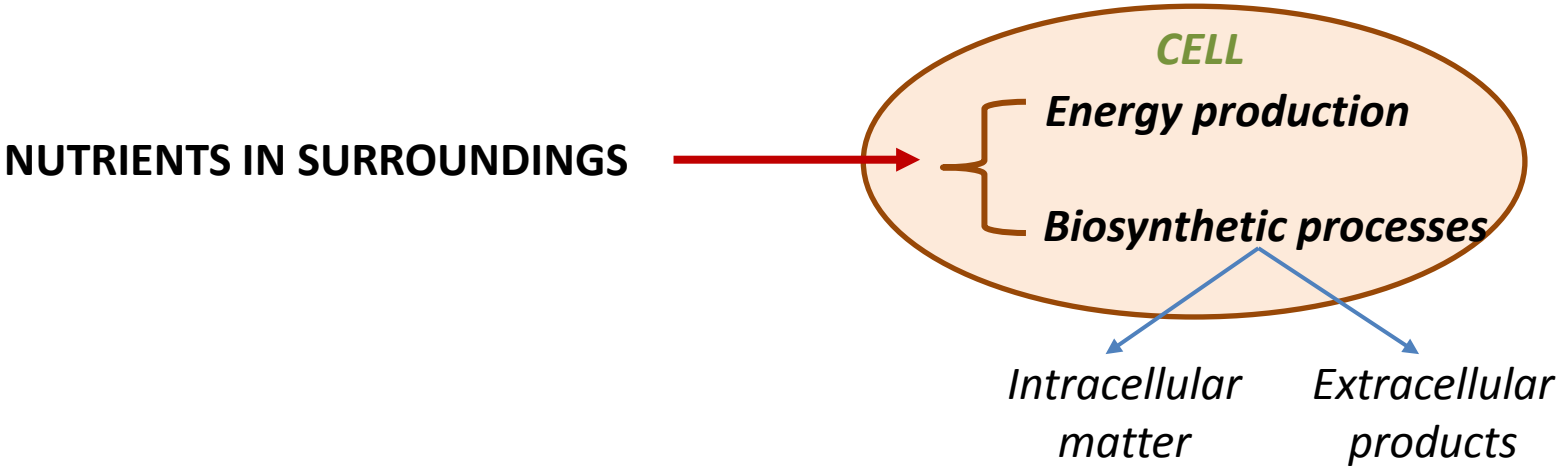
CELL GROWTH IN BATCH SYSTEMS

The growth and proliferation of cells is the ***basic function*** of living organisms.

GROWTH	=	CELL REPLICATION =***PROLIFERATION***	+	CHANGE OF CELL SIZE

To keep growing, the cells need to take **nutrients** from the surroundings and change them to **cellular matter** (**biomass**) and **energy**:



The mass of cells present in a space containing nutrients is growing in time = ***growth.***
Symbolic description of growth:

substrates/nutrients + cells/biomass → extracellular products + more cells

$$\sum_i S_i + X \longrightarrow n\,X + \sum_j P_j\,, \quad n > 1$$

(autocatalytic process)

Quantification of cell growth: (measurement, modelling, calculations...)

- ***SPECIFIC GROWTH RATE:*** $$\mu = \frac{1}{X}\frac{\mathrm{d}X}{\mathrm{d}\tau}$$ (h^{-1})

X ... concentration of cells in the environment (commonly in g dm^{-3},i.e., in mass concentration units) – other units may be used, such as moles dm^{-3} (which is less practical)

τ ... time of growth (commonly in hours)

The key question is, how the specific growth rate is influenced by the properties of the environment in which the cells are present?

Cell growth → in ***batch systems*** (unsteady process)

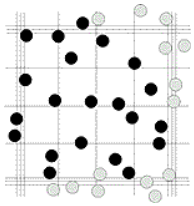
Cell growth → in ***continuous systems*** (steady state can be reached)

Determination of cell concentration in the environment (the cultivation medium)

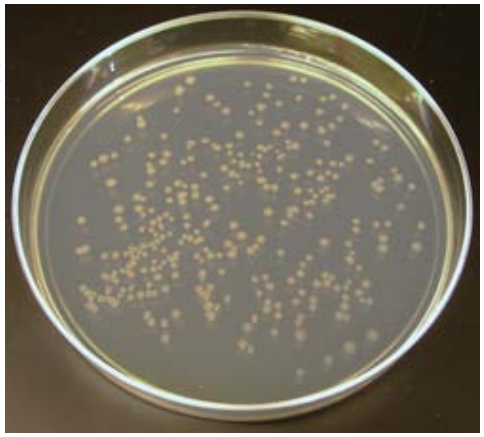
- knowing cell concentration is the key for describing ***growth kinetics*** and its ***stoichiometry***
- **methods**: ***direct*** (number or weight of cells) and ***indirect*** (weight or concentration only of a certain compound of the biomass, e.g. protein or nucleic acid, is quantified)

Cell number quantification:

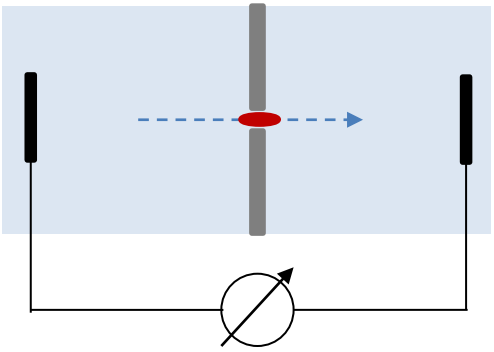
a) in **a counting chamber** under a microscope (Bürker's chamber) – it is a special microscopic slide with a raster. A suspension of known volume is inserted into the chamber and the cells are counted inside the individual raster fields. Knowing the dilution factor of the suspension, the original cell concentration can be calculated.



b) **counting of colonies** grown on agar plates after inoculation / seeding with a suitably diluted culture of cells. When using this method it is expected, that from each single cell in the original suspension grows exactly one colony.



b) **counting cells** using cell counters. Two chambers connected only by a small opening are filled with electrolyte. When a cell enters the channel, the cell volume pushes out electrolyte from the channel, which results in a change in the electric resistance of the system. Each rise in the resistance is counted.



d) **nefelometry** – measuring light scattering on the cells in suspension

e) **measuring optical density** of cell suspension (light attenuation)

Measuring the cell mass: cells are separated from the cultivation medium using centrifugation or filtration and after washing they are dried at 105 °C until constant weight is reached. The cell concentration is then expressed as ***the mass of the dried cells in a certain volume of the original sample***, most commonly in grams of dried biomass per 1 dm^3 of culture (drying is performed under specified conditions until constant weight is obtained, typically 12 h at 105°C).

INDIRECT METHODS: for example the concentration of RNA, DNA, some specific proteins, amino acids, intracellular ATP, amount of produced CO_2 , or viscosity of the cultivation medium are measured

CELL GROWTH IN BATCH CULTURES

(liquid media)

- After ***inoculation*** of the cultivation medium by a small amount of cells – called ***inoculum*** – the cell culture starts to grow
- The growth consists of several phases:

  a) ***Lag phase*** – the cells are adapting to the new environment, for example new enzyme systems are being activated, i.e., cells are not replicating themselves. The length of the lag phase is strongly influenced by the physiological state of the inoculum. If more than one medium is present, several lag phases can successively occur during the cultivation (diauxic growth).

  b) ***Exponential / logarithmic phase*** – fast replication of cells is occurring. The number of cells is growing exponentially and the size of the cells is growing as well. It is a period of balanced growth, when all of the cell components are growing at the same rate. The composition of the biomass stays relatively constant. The specific growth rates measured by the cell number or cell mass gives the same results (***balanced growth***). In this phase, the concentration of nutrients in the cultivation medium is high so that the growth is not limited by the nutrient concentration and can be described using **first order kinetics**:

$$\frac{\mathrm{d}X}{\mathrm{d}\tau} = \mu X, \quad (\tau = 0 : X = X_0) \qquad \rightarrow \qquad X = X_0 \exp(\mu\tau)$$

  For μ = const this equation predicts unlimited exponential growth!!!
  Generation/doubling time: time, after which the amount of biomass is doubled: $\tau_\mathrm{d} = \ln 2 / \mu$

  Primary metabolites: are produced within cells (e.g., proteins) only when the cells are proliferating.

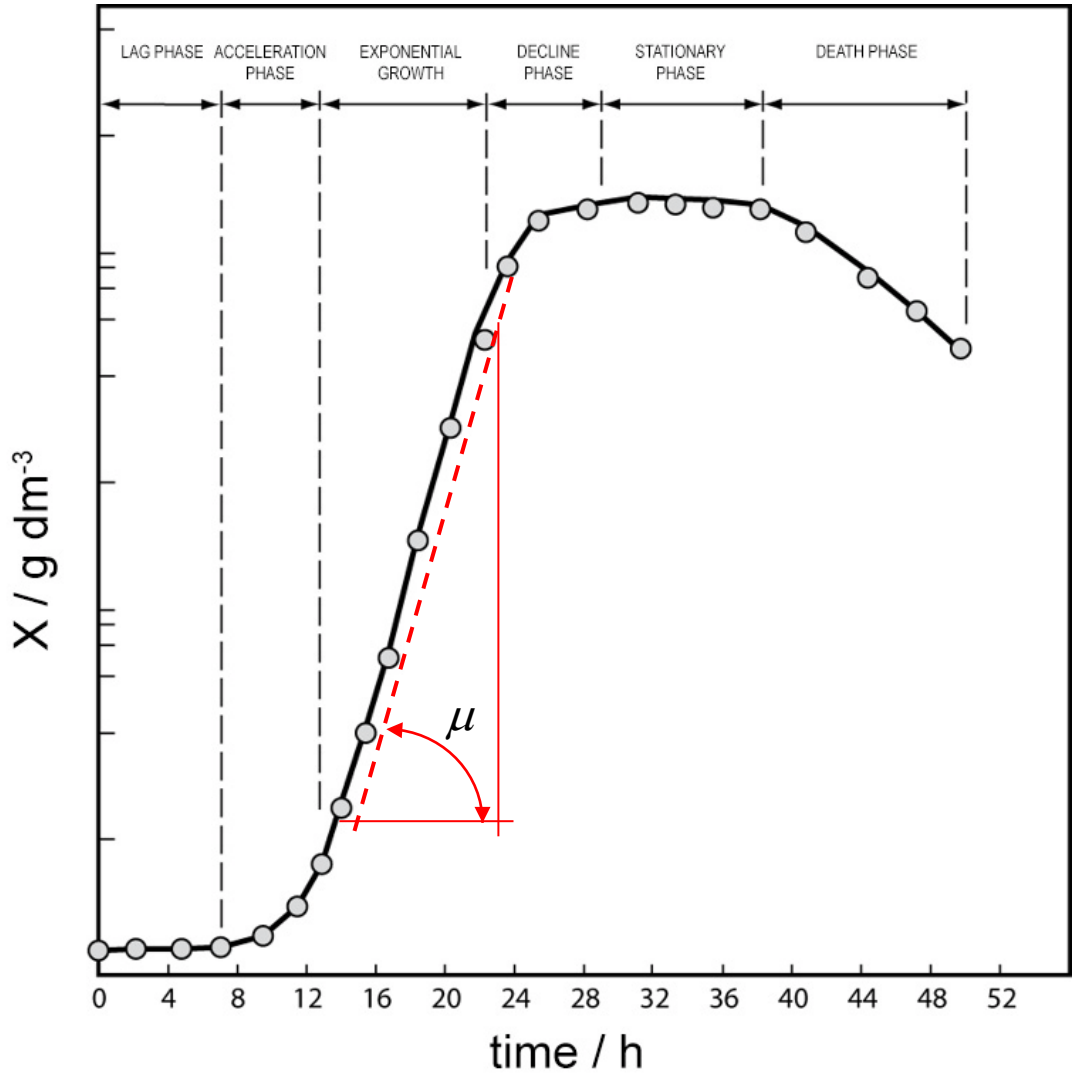
  c) ***Growth deceleration phase*** – slowing down of growth is the outcome of depleting one of the nutrients in the medium or accumulation of toxic (growth inhibiting) products or metabolites in the medium (for example ethanol). Notable changes in the composition of biomass occur – phase of ***unbalanced growth***.

  d) ***Stationary growth phase*** – the total growth rate is zero, because the cells are either not replicating or the cell division rate equals to the cell death rate. The cells are still active and produce **secondary metabolites** (those that are not tied to the cell growth).

Graphical representation of the dependence of biomass growth on time – the **growth curve** of microorganism.

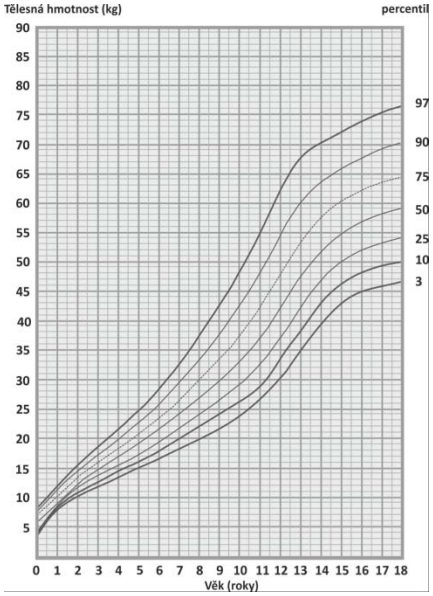
The values on the Y axis are in logarithmic coordinates.

The slope of the linear part in the exponential phase gives us the specific growth rate value.



Development of a typical growth curve of a microorganism in a liquid medium.

A comparison - typical growth curve of a "macroorganism" (man)



In the stationary phase:

i. the total weight of the cells might stay constant, but the number of live cells might be declining
ii. the cells may undergo lysis and the number of live cells is declining. Additional growth phase can occur using the lysis products from the dead cells (cryptic growth)
iii. cells are not growing anymore, but their metabolism is still active and the cells produce secondary metabolites.

e) ***death phase*** – follows the stationary phase, but the transition is slow and not very clear. In this phase the cells undergo lysis and release nutrients for growth of other cells

Cells death rate:

$$\frac{\mathrm{d}N}{\mathrm{d}\tau} = -k_d^{'} N \,,$$

$$N = N_{st.} \exp\left(-k_d^{'} \tau\right).$$

where $N_{st.}$ is the number of cells (concentration) in the medium at the end of the stationary phase and k_d is the kinetic constant of death occurances (h^{-1}). The k_d value depends on temperature according to the Arrhenius equation. When modelling the cell death dynamics, using their mass is not suitable, since even dead cells have non-zero mass.

STOICHIOMETRIC PARAMETERS OF CELL GROWTH

- The cells convert substrate(s) to biomass, extracellular products and energy. These processes can be formally understood as simple chemical reactions and be described using quantities analogous to stoichiometric coefficients in chemical equations.
- Stoichiometric parameters are useful for the description of biomass growth and the product production, for balancing the amount of substrates, biomass and products and for evaluating experiments.

- **The growth yield for substrate (the yield coefficient)**: gives the amount of biomass, which is created from 1 kg of consumed substrate

$$Y_{X/S} = \frac{X - X_0}{S_0 - S} = -\frac{\Delta X}{\Delta S} \approx -\frac{\mathrm{d}X}{\mathrm{d}S}$$

Typical values of $Y_{X/S}$: **0,4 – 0,6** (kg X/kg S)

Substrate is consumed for biomass growth, production of extracellular products, production of energy needed for growth (typically chemically bound energy in macroergic compounds, e.g. ATP) and for so called maintenance energy:

$$\Delta S = \Delta S_{\text{Assimilated into biomass}} + \Delta S_{\text{Assimilated into extracellular products}} + \Delta S_{\text{Assimilated into energy for growth}} + \Delta S_{\text{Assimilated into maintenance energy}}$$

The value of the growth yield may be changing in time during the growth (because different metabolic pathways may be overlapping). The yield calculated from the whole period of culture growth is called ***the total/apparent growth yield*** which expresses stoichiometry of the overall metabolism***.***

- **Other yield coefficients:**

  a) Yield of biomass per consumed oxygen: $Y_{X/O_2} = -\frac{\Delta X}{\Delta O_2}$ $Y_{X/02}$ = (0,9 – 1,4) kg X/kg O_2

  b) Yield of product per consumed substrate: $Y_{P/S} = -\frac{\Delta P}{\Delta S}$

  c) The maintenance coefficient: $m = \frac{(dS/d\tau)_m}{X}$ for describing the stationary phase of the growth curve. It is a specific rate of substrate consumption needed for maintaining physiological state of the cell.

MICROBIAL PRODUCTS created by cells during growth:

1) ***Primary products tied to the cell growth*** – specific production rate of these products is proportional to the cell specific growth rate, for example constitutive enzymes:

specific rate of product production: $q_P \equiv \frac{1}{X}\frac{dP}{d\tau} = Y_{P/X}\mu$ [kg P/kg X/h]

2) ***Secondary products not tied to the cell growth*** – formed mostly during the stationary phase of the growth curve, when the cell growth rate is zero. The specific rate of product production is constant:

$$q_P \equiv \beta = const.$$ [kg P/kg X/h]

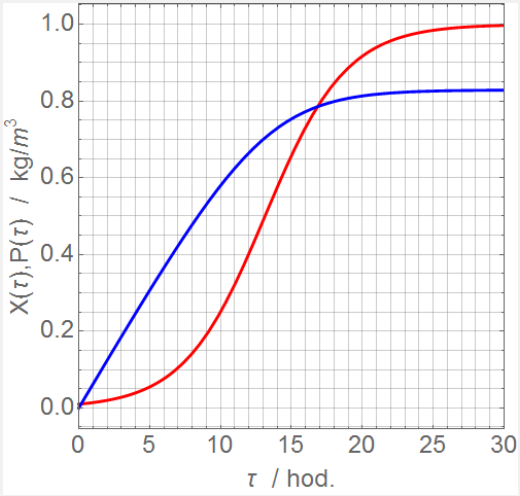
3) ***Mixed products with partial tie to the cell growth*** – formed during the decelerating phase and during the stationary phase (for example the lactic acid):

Luedeking-Piret equation

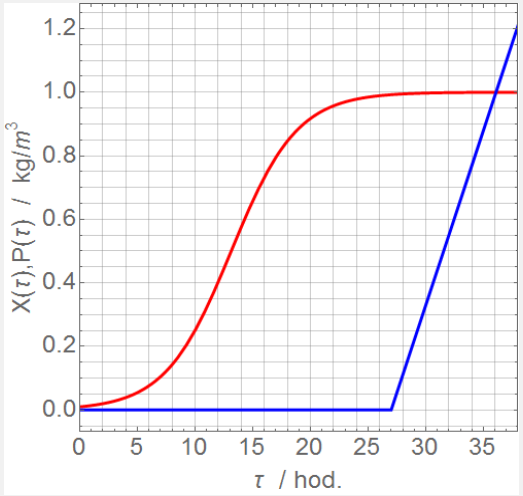
$$q_P \equiv \alpha\,\mu + \beta$$ [kg P/kg X/h]

□, □ are parameters

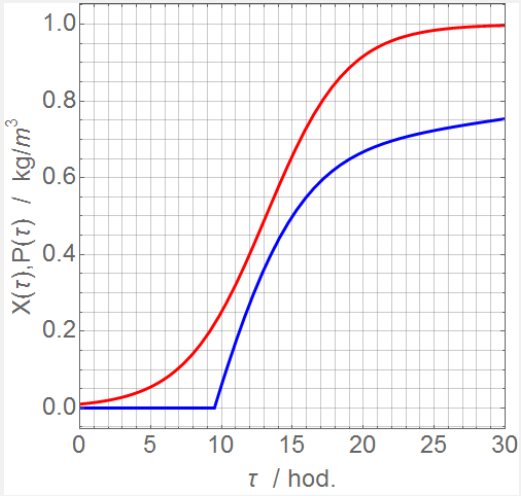
Development of produced amount of product P (blue) and biomass X (red) in time for different microbial product types



Product tied to growth



Product not tied to the growth



Product partially tied to growth

EFFECT OF CULTIVATION CONDITIONS ON THE CELL GROWTH KINETICS

TEMPERATURE

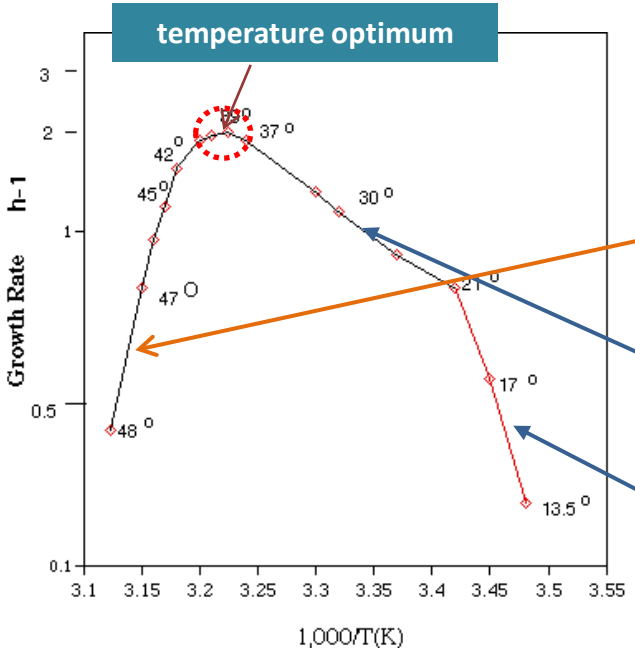
- Depending on the temperature requirements, microorganisms are divided to **psychrofiles**, **mesofiles** and **thermofiles** – as was mentioned earlier.
- The cell growth doubles when the temperature rises by 10 °C, after breaching the optimal temperature, the growth starts to decline which is followed by ***the cell thermal death*** (denaturation of enzymes and other unstable compounds).
- **Total growth rate:**

$$\frac{dX}{d\tau} = \left(\mu - k_d^{'}\right) X$$

$$\mu = A \exp\left(-\frac{E_a}{RT}\right) \qquad k_d^{'} = A^{'} \exp\left(-\frac{E_d}{RT}\right)$$

E_a = 80 – 160 kJ/mol

E_d = 240 – 320 kJ/mol



Arrhenius chart **(ln μ vs 1/T)** ***shows the dependence of specific growth rate on temperature***

At high temperatures the thermal death of cells occurs (inactivation)

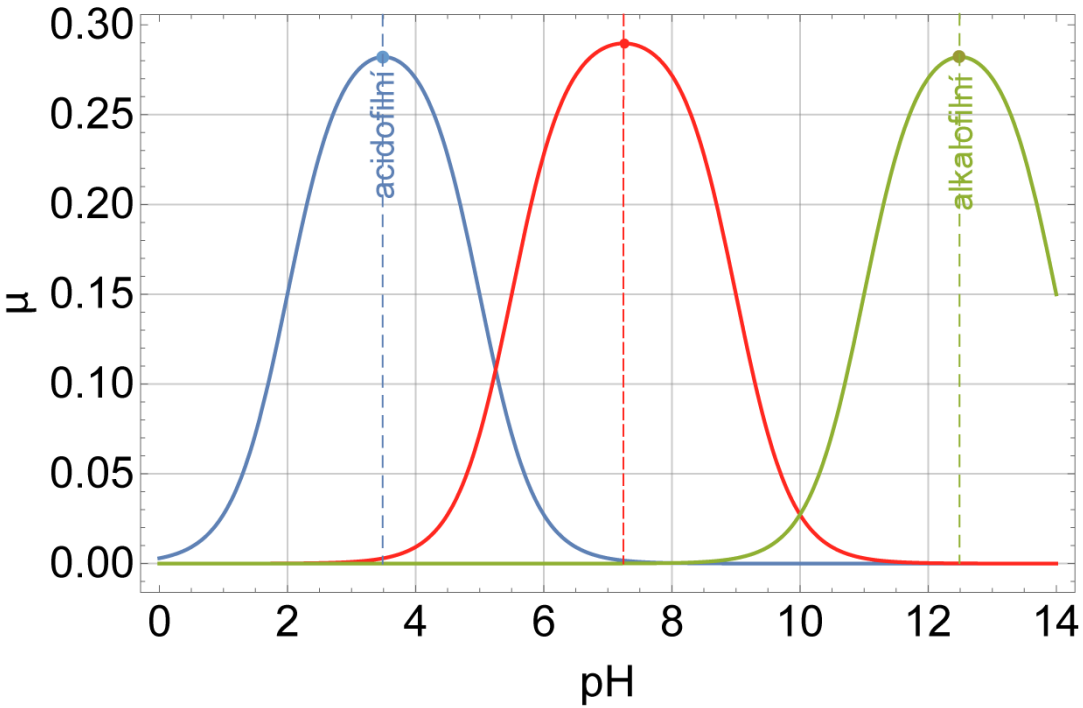
At medium temperatures, the rise of the growth rate slows down, because the (slow) transport of nutrients through the cell membrane becomes the limiting factor of growth

At low temperatures, the growth rate is rising (is being activated) with rising temperature

- **Product production rate** depends on the temperature similarly as the cell growth, but the temperature optimums is generally varies.

EFFECT OF pH

- The reaction rate of enzyme catalysed reactions in live cells is dependent on the pH. That way also the growth of cells depends on the pH of the environment, in which they live.
- Microorganisms generally differ in their optimal pH values (pH_{opt}) - the range of pH where the growth rate is the highest. Most of the microorganisms have the optimum in the neutral pH range, but also some **acidophilic** and **alkalophilic** bacteria exist**:**



- Microorganisms generally have some ability of adapting to different pH values. The dependences in the chart can thus be slightly altered by changing the pH of the cultivation medium slowly.

- Optimal pH values for cell growth and for the metabolite production can differ.
- pH of the cultivation medium can change (even drastically) during the cultivation:
    - In the metabolic processes **organic acids** are formed from **sugars** – **the pH of the medium drops** (e.g. during fermentation production of the citric acid),
    - Ammonia forms by **deamination of aminoacids** – **the pH of the medium rises**.
- Cultivation media thus must have some **buffering capacity** or it is necessary to continuously **titrate** the medium by adding bases or acids.

EFFECT OF DISSOLVED OXYGEN CONCENTRATION

- During **aerobic cultivations** the concentration of oxygen can be the limiting factor of growth and metabolite production, because the **solubility of oxygen** in water media is **low**.
- At **high biomass concentrations** in the medium the oxygen consumption rate can overcome its transfer rate into the medium and **the concentration of oxygen in the medium can drop under a critical value** $c_{02,crit.}$ (see the chart on slide 13) and the cells may begin to die, or their metabolism can strongly change!
- The critical concentration of dissolved oxygen is usually about 10% of the saturated concentration of O_2 in the solution.
- The solubility of oxygen in water at 25 °C and pressure of 101 kPa is about 8 ppm (8.4 mg/dm^3).
- **The oxygen transfer rate** into the medium (OTR)

$$J_{O_2} = k_L a \left(c_L^* - c_L \right)$$

where J_{O2} is the molar flow of oxygen between the gas and liquid phase, *a is the density of interphase area and* c_L^*, c_L *are* concentrations of oxygen in the saturated solution (in a liquid in a close vicinity of the phase

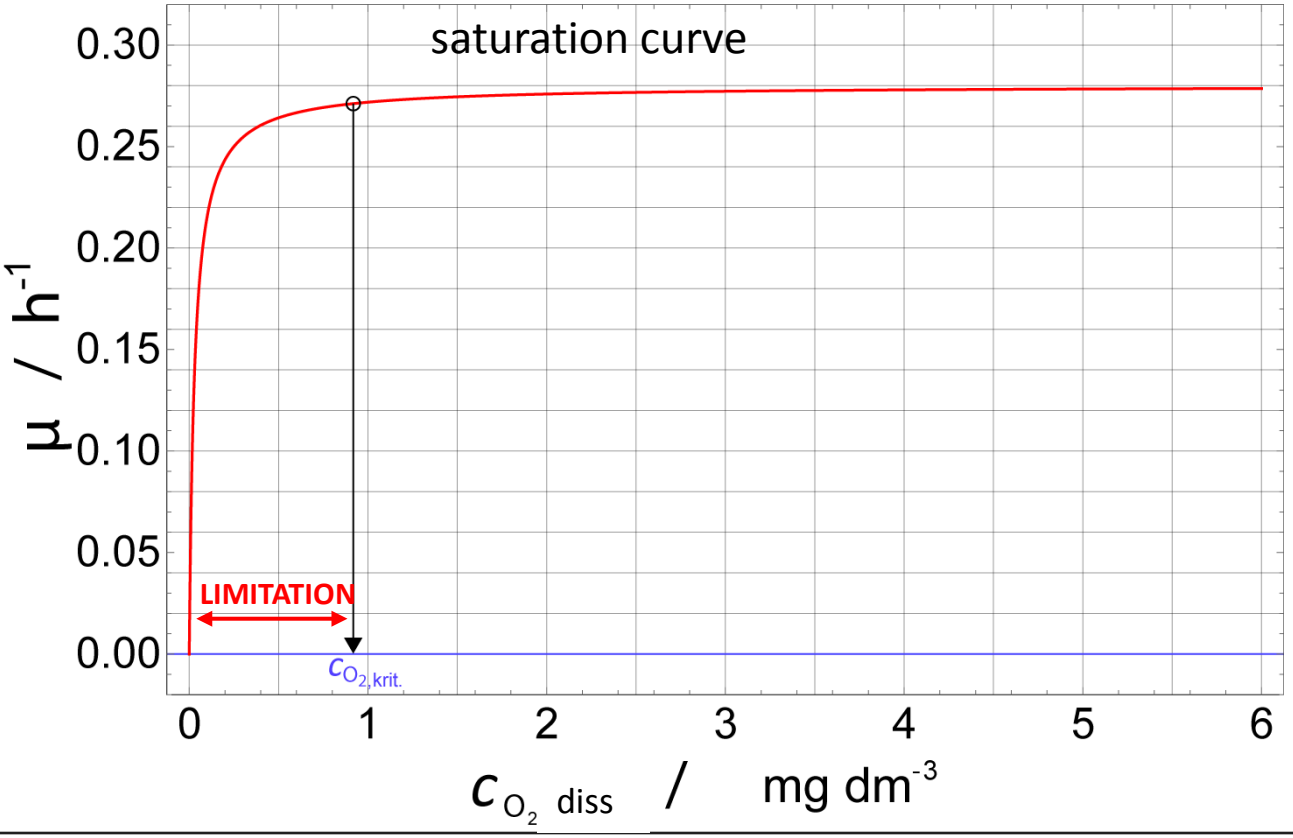
interphase) and its current concentration in the medium and k_L is the mass transfer coefficient between the gas and the liquid phase (its value can be influenced by stirring or by the flow rate of the liquid medium). The equilibrium oxygen concentration can be increased by increasing the oxygen amount in the gas phase.

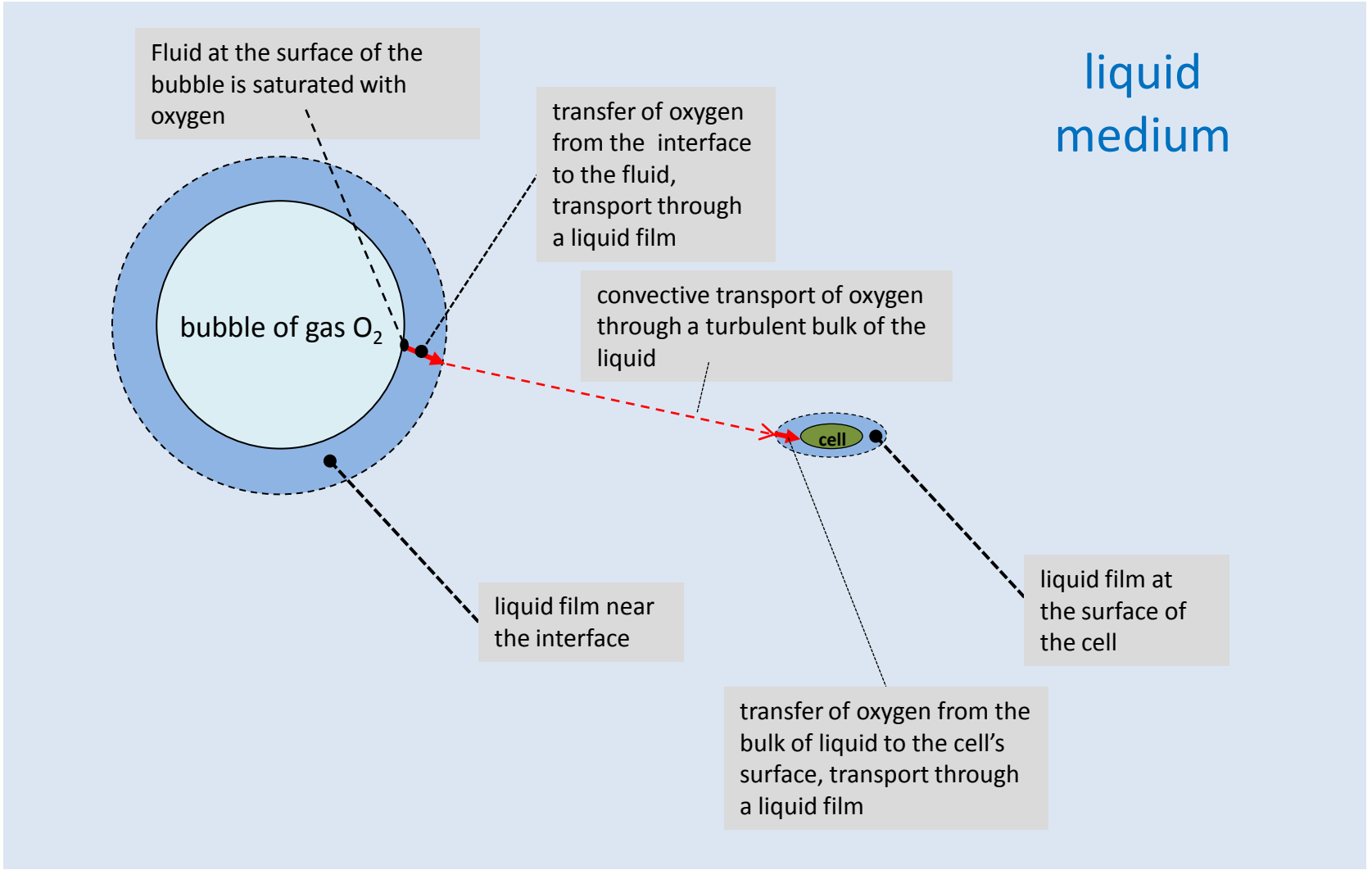
Oxygen uptake rate by cells of microorganisms (OUR)

$$J_{O_2} = q_{O_2} X = \mu \frac{X}{Y_{X/O_2}}$$

where q_{O2} is the specific rate of oxygen uptake by the microorganism (mg O_2 per mg of biomass per 1 hour) and $Y_{X/O2}$ is the growth rate of the microorganism for oxygen

Dependence of specific growth rate on the dissolved oxygen concentration





Transport processes in aerobic cultivations

Limitation of growth by oxygen: occurs, when the transfer rate of oxygen from the gas into the cultivation medium is lower or equal to the rate of its uptake:

$$\mu \frac{X}{Y_{X/O_2}} \geq k_L a \left(c_L^* - c_L \right)$$

if we express the specific growth rate using its definition, we get the following expression :

$$\frac{\mathrm{d}X}{\mathrm{d}\tau} = Y_{X/O_2} k_L a \left(c_L^* - c_L \right) \quad ,$$

which shows that when the growth is limited by oxygen, the cell growth is directly proportional to the transfer rate of oxygen from gas to liquid.

Other factors affecting the cell growth:

a) *redox potential of the medium*,
b) *ionic strength of the medium*,
c) concentration of dissolved carbon dioxide,
d) *osmotic pressure of the medium*.

Modelling the cell growth

Mathematical models of cell growth describe the dependence of the growth rate on the physico-chemical conditions under which the growth occurs. When modelling cell growth, one should take into account, that the growth is generally unbalanced, meaning that the composition of biomass changes during the growth period, and that the cell population includes individuals in various physiological states (varying age). Models including all of these factors are very complex and not very convenient for practical operations. For that reason, simplified models are often used, despite being less precise.

Structured models of growth: the cells are described in terms of functional compartments. Cell = SUM(proteins, nucleic acids, ATP ...)

Segregated models of growth: the cells are described in different stages of their life cycle (various ages), the composition of the cell population is taken into account.

Possible model combinations:

1) **UNSTRUCTURED and UNSEGREGATED MODEL**
2) **STRUCTURED and UNSEGREGATED MODEL**
3) **UNSTRUCTURED and SEGREGATED MODEL**
4) **STRUCTURED and SEGREGATED MODEL**

complexity of the models grows

In this course we will deal **with only models from the first group** (and not too detailed).

The general question is, how does the specific growth rate depend on the substrate concentration.

The most often used dependence of specific growth rate on the substrate concentration is the ***Monod equation*** (semi-empirical model), which describes well the microorganism growth in most cases:

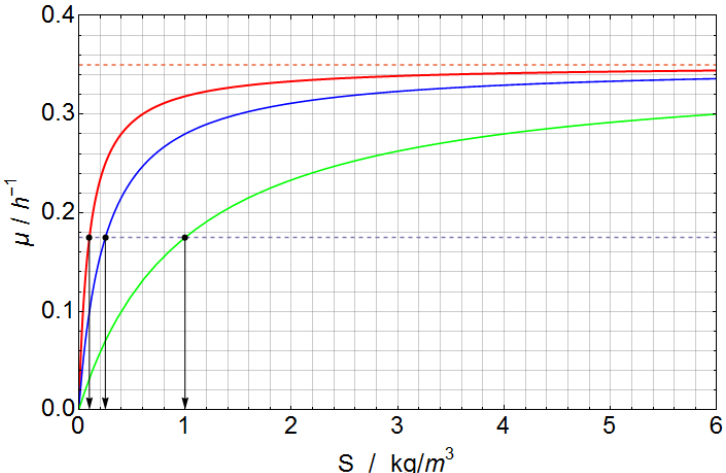
$$\mu = \frac{\mu_{max} S}{K_s + S}$$

S is concentration of substrate limiting the growth, so called *limiting substrate*

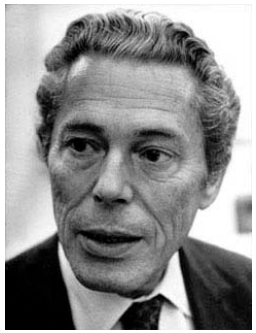
μ_{max} is ***maximum growth rate*** of the microorganism (h^{-1}),

K_s is ***saturation constant*** which is equal to the substrate concentration, at which the growth rate is half of the maximum growth rate value

Monod kinetics is an analogy to the Michaelis-Menten kinetics due to enzymes in the cells



Monod dependences of the specific growth rate on the concentration of the substrate at three different values of the saturation constant



Jacques Monod
9.2.1910 – 31.5.1976
Nobel prize in 1965

Monod equation well describes slow growth of cells at low concentrations. For ***fast growth in concentrated cultures*** for example these models are being used:

$$\mu = \frac{\mu_{\max} S}{K_{S_0} S_0 + S} \qquad \text{or} \qquad \mu = \frac{\mu_{\max} S}{K_{S1} + K_{S_0} S_0 + S}$$

which also accounts for the effect of initial substrate concentration S_0.

Alternative growth models:

1) *Blackman model* (contains discontinuity!)

$$\mu = \mu_{\max} \quad \text{pro} \quad S \geq 2K_S$$
$$\mu = \frac{\mu_{\max} S}{2K_S} \quad \text{pro} \quad S < 2K_S$$

2) *Tessier model*

$$\mu = \mu_{\max}\left(1 - e^{-KS}\right)$$

3) *Moser model*

$$\mu = \frac{\mu_{\max} S^n}{K_S + S^n}$$

4) *Contois model*

$$\mu = \frac{\mu_{\max} S}{K_{SX} X + S}$$

Growth of cells in the presence of several substrates:

1) *Interactive/multiplicative model model*

$$\frac{\mu}{\mu_{\max}} = \mu(S_1) \cdot \mu(S_2) \cdot \ldots \cdot \mu(S_{n-1}) \cdot \mu(S_n)$$

2) *Additive model*

$$\frac{\mu}{\mu_{\max}} = w_1\mu(S_1) + w_2\mu(S_2) + \ldots + w_{n-1}\mu(S_{n-1}) + w_n\mu(S_n)$$

, where w_i are weight functions.

If all growth rates are of Monod type, then $w_i = \frac{K_i/S_i}{\sum_{j=1}^{n} K_j/S_j}$

3) *Non-interactive model* $\mu = \mu(S_1) \vee \mu(S_2) \vee \ldots \vee \mu(S_{n-1}) \vee \mu(S_n)$, where $\mu(S_i)$ is chosen with the lowest value.

Predictive ability of these models may not be high - it is required to evaluate each case individually and compare the model with experimental results.

Cell growth in the presence of inhibitors

- Inhibition (growth retardation) can be caused by: ***substrates***, ***products*** or ***inhibitors***.
- Inhibition constants in kinetic equations (they are analogous to the equation used for enzymatic reactions) have **physiological importance** if the growth of the affected cells is governed only by one enzymatic reaction. In other cases they are simply numerical constants with no physical meaning.
- ***Description of growth inhibition by substrate:***

  a) *competitive inhibition by substrate* $\mu = \frac{\mu_{max} S}{K_S\left(1 + S/K_I\right) + S}$

  b) *non-competitive inhibition by substrate* $\mu = \frac{\mu_{max}}{\left(1 + K_S/S\right)\left(1 + S/K_I\right)}$

  K_I is inhibition constant for substrate, K_P is inhibition constant for product

- ***Description of growth inhibition by product:***

  a) *competitive inhibition by product* $\mu = \frac{\mu_{max} S}{K_S\left(1 + P/K_P\right) + S}$

  b) *non-competitive inhibition by product* $\mu = \frac{\mu_{max}}{\left(1 + K_S/S\right)\left(1 + P/K_P\right)}$

  An example is yeast inhibition by ethanol during fermentation (>5%)

For the description of *cell growth inhibition by ethanol* also these equations are used:

$$\mu = \frac{\mu_{max}}{1 + K_S/S}\left(1 - \frac{P}{P_m}\right)^n$$

P_m … limiting concentration of EtOH, when the growth stops

$$\mu = \frac{\mu_{max}}{1 + K_S/S}\exp\left(-\frac{P}{K_P}\right)$$

Description of growth inhibition by toxic substances:

Equations analogous to the enzyme inhibition equations are used:

- *competitive inhibition of growth*

$$\mu = \frac{\mu_{max} S}{K_S\left(1 + I/K_I\right) + S}$$

- *non-competitive inhibition of growth*

$$\mu = \frac{\mu_{max}}{\left(1 + K_S/S\right)\left(1 + I/K_I\right)}$$

- *acompetitive inhibition of growth*

$$\mu = \frac{\mu_{max} S}{\left(\frac{K_S}{1 + I/K_I} + S\right)\left(1 + \frac{I}{K_I}\right)}$$

However, it is questionable, how much are these equations valid for cell growth. Caution is required when using them. Even if the experimental data are well fitted using one of these equations, it does not necessarily mean, that the inhibition mechanism is actually the one, for which the equation was derived. It is necessary to conduct several independent experiments and thus have multiple data sets available for analysis. Especially, if the experimental errors are high.

Logistic equation: description of cell growth by combining the Monod kinetics and balancing the biomass using the growth yield (thus the substrate concentration S is eliminated from the biomass balance *X*)

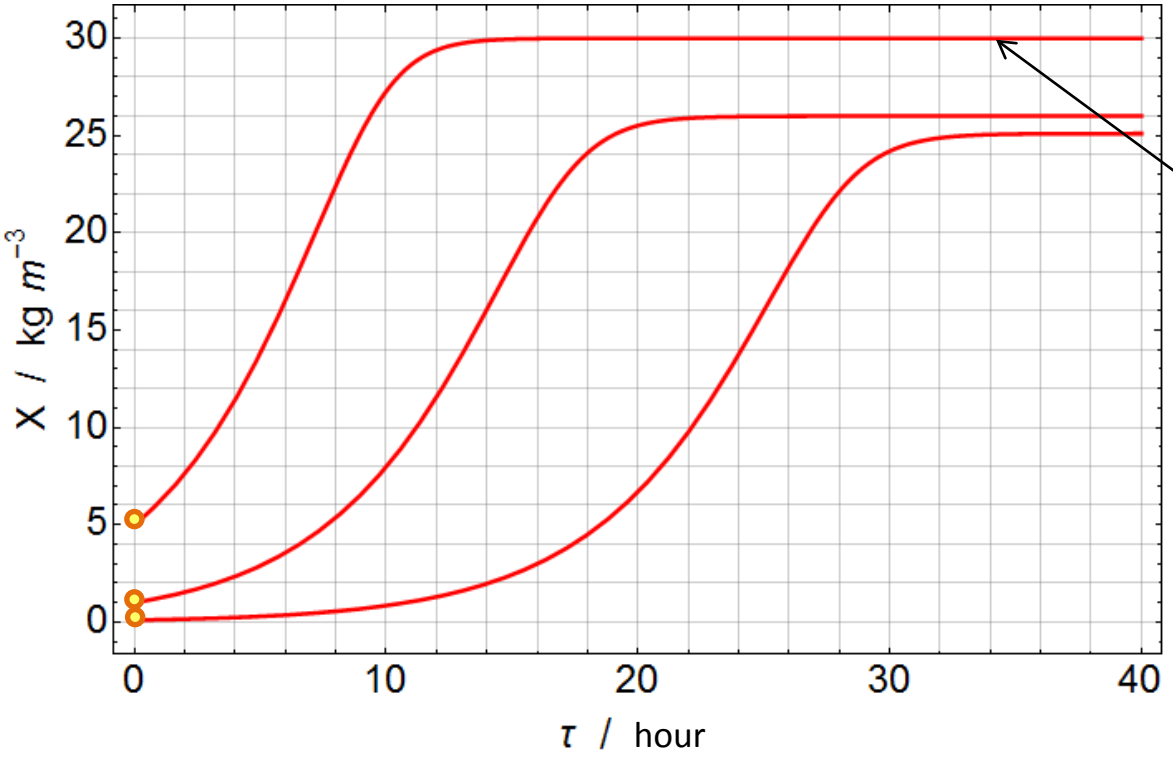
In a batch system:

$$\frac{dX}{d\tau} = \mu X = \frac{\mu_{max} S}{K_S + S} X$$

$$S = S_0 - \frac{X - X_0}{Y_{X/S}}$$ from growth yield definition

$$\frac{dX}{d\tau} = \frac{\mu_{max}\left(S_0 - \frac{X - X_0}{Y_{X/S}}\right)}{K_S + \left(S_0 - \frac{X - X_0}{Y_{X/S}}\right)} X = \frac{\mu_{max}\left(Y_{X/S} S_0 - X + X_0\right)}{Y_{X/S} K_S + Y_{X/S} S_0 - X + X_0} X$$

By numerical solution of the last equation we recieve the dependence of concentration of cells on time:



Maximum biomass concentration
$X_\infty = X_0 + Y_{X/S}\, S_0$
(carrying capacity of environment)

The curves differ only in the initial cell concentration value S_0

The logistic curve can also be reached via an alternative route, without the need of Monod equation. Instead the following specific growth rate dependence is used:

$$\mu = k\left(1 - \frac{X}{X_\infty}\right)$$

The growth rate is directly proportional to the unused environmental capacity and is not dependent on the substrate concentration. Then:

$$\frac{\mathrm{d}X}{\mathrm{d}\tau} = \mu X = k\left(1 - \frac{X}{X_\infty}\right) X$$

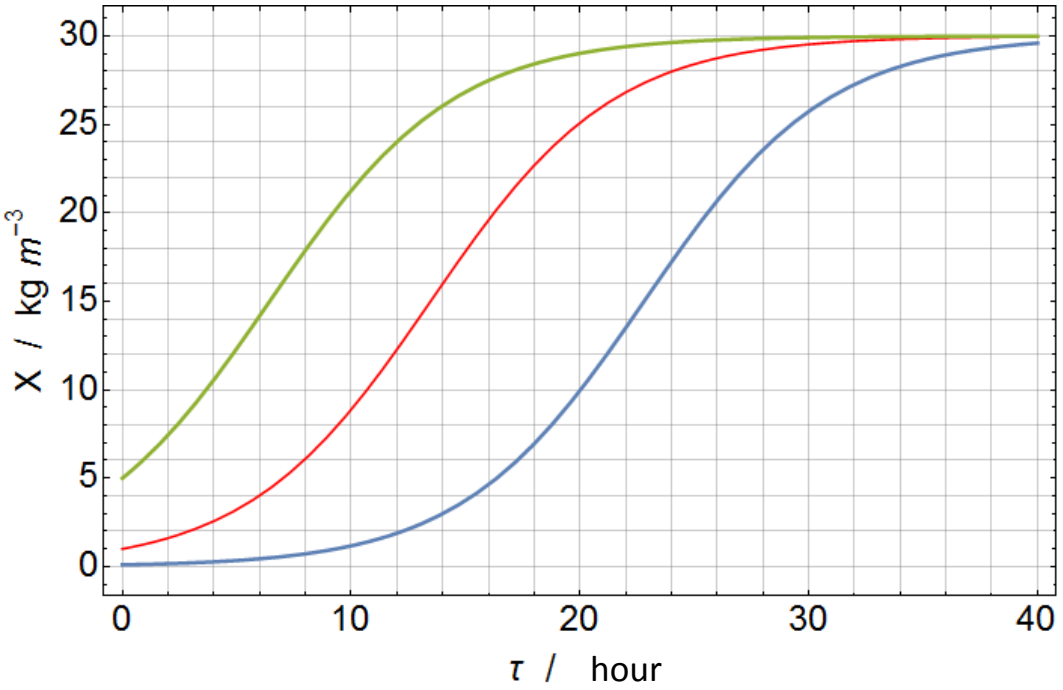
After separation of unknowns we obtain:

$$\int_{X_0}^{X} \frac{\mathrm{d}X}{\left(1 - X/X_0\right) X} = \int_0^\tau k\, \mathrm{d}\tau$$

And after integration and expression of X:

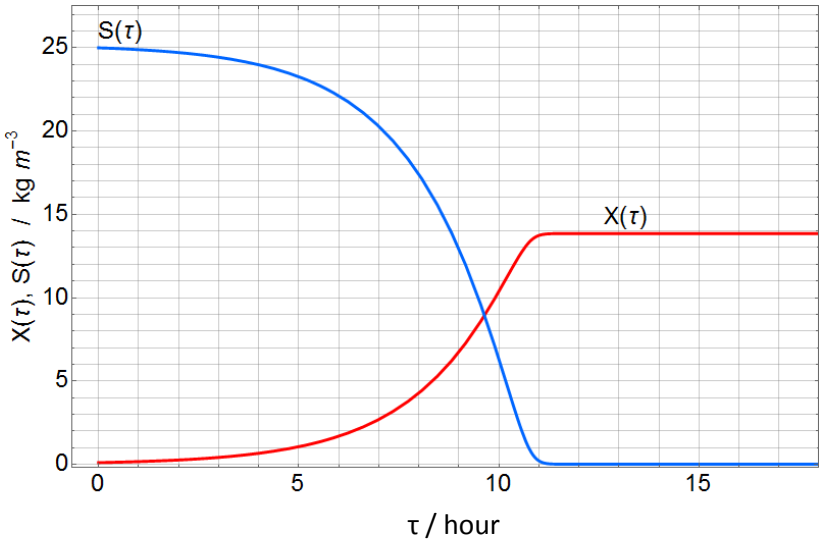
$$X = \frac{X_0 \exp(k\tau)}{1 - \frac{X_0}{X_\infty}\left[1 - \exp(k\tau)\right]}$$

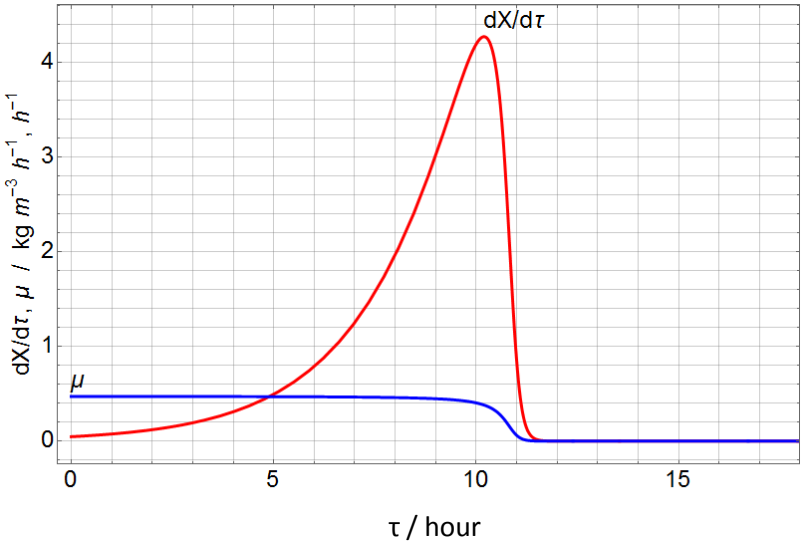
The evolution of biomass concentration in time is shown for three different initial cell concentrations:



The curves in this graph are in principle similar to those in the previous graph. However, the differences in the carrying capacity of the environment are apparent (the X_∞ values). In this case, the X_∞ is one of the model parameters. In the previous case it was a function of other parameters in the model: $X_\infty = X_0 + Y_{X/S}\, S_0$. The form of the logistic equation shown above is used to describe the growth of populations mainly in ecology.

Numerical modelling of cell growth in a batch system - 1





The dependence of cell concentration X and concentration of substrate S on the cultivation time τ can be obtained by:

a) solving a system of two differential equations – by balance equations for the cell mass and the mass of substrate:

$$\frac{\mathrm{d}X(\tau)}{\mathrm{d}\tau} = \mu X(\tau) = \frac{\mu_{\max} S(\tau)}{K_S + S(\tau)} X(\tau),$$

$$\frac{\mathrm{d}S(\tau)}{\mathrm{d}\tau} = -\frac{\mu}{Y_{X/S}} X(\tau) = -\frac{1}{Y_{X/S}} \frac{\mu_{\max} S(\tau)}{K_S + S(\tau)} X(\tau),$$

$$S(0) = S_0,\ X(0) = X_0$$

b) Substituting for S in the biomass balance using the growth yield $Y_{X/S}$ (which follows from summing balances of S and X) and by solving the sole ordinary differential equation:

$$\frac{\mathrm{d}X(\tau)}{\mathrm{d}\tau} = \mu X(\tau) = \frac{\mu_{\max}\left[S_0 - \frac{X[\tau]-X_0}{Y_{X/S}}\right]}{K_S + \left[S_0 - \frac{X[\tau]-X_0}{Y_{X/S}}\right]} X(\tau),$$

$$X(0) = X_0$$

Numerical modelling of cell growth in a batch system – 2

(solution in the software Mathematica® 11)

a)

```
In[129]:= mumax = .5;
          Ks = 1.5;
          yxs = 0.55;
          s0 = 25;
          x0 = 0.1;
          tmax = 18;

In[135]:= eqns = {x'[t] == mumax s[t]/(Ks + s[t]) x[t], s'[t] == -1/yxs mumax s[t]/(Ks + s[t]) x[t], x[0] == x0, s[0] == s0};

In[136]:= d = NDSolveValue[eqns, {x, s}, {t, 0, tmax}]

Out[136]= {InterpolatingFunction[Domain: {{0., 18.}} Output: scalar], InterpolatingFunction[Domain: {{0., 18.}} Output: scalar]}
```

b)

```
In[139]:= eqns2 = {x1'[t] == mumax (s0 - (x1[t]-x0)/yxs)/(Ks + s0 - (x1[t]-x0)/yxs) x1[t], x1[0] == x0}

Out[139]= {x1'[t] == 0.5 (25 - 1.81818 (-0.1 + x1[t])) x1[t]/(26.5 - 1.81818 (-0.1 + x1[t])), x1[0] == 0.1}

In[140]:= x1 = NDSolveValue[eqns2, x1, {t, 0, tmax}]

Out[140]= InterpolatingFunction[Domain: {{0., 18.}} Output: scalar]
```

Dependence of growth rate and specific growth rate on the cultivation time:

```
In[143]:= Plot[{mumax (s0 - (x1[t]-x0)/yxs)/(Ks + s0 - (x1[t]-x0)/yxs) x1[t], mumax (s0 - (x1[t]-x0)/yxs)/(Ks + s0 - (x1[t]-x0)/yxs)}, {t, 0, tmax}, PlotRange → {{0, tmax}, All}, PlotStyle → {{Red, Thickness[0.004]}, {Blue, Thickness[0.004]}},
  Frame → True, FrameLabel → {"τ  / hod.", "dX/dτ, μ  /  kg m^-3 h^-1, h^-1"}, ImageSize → 800, GridLines → Full, LabelStyle → {Large, Black},
  PlotLabels → Placed[{"dX/dτ", "μ"}, Above]]
```