Bioengineering I
Winter semester 2022
The selected chapters

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EUROPEAN UNION
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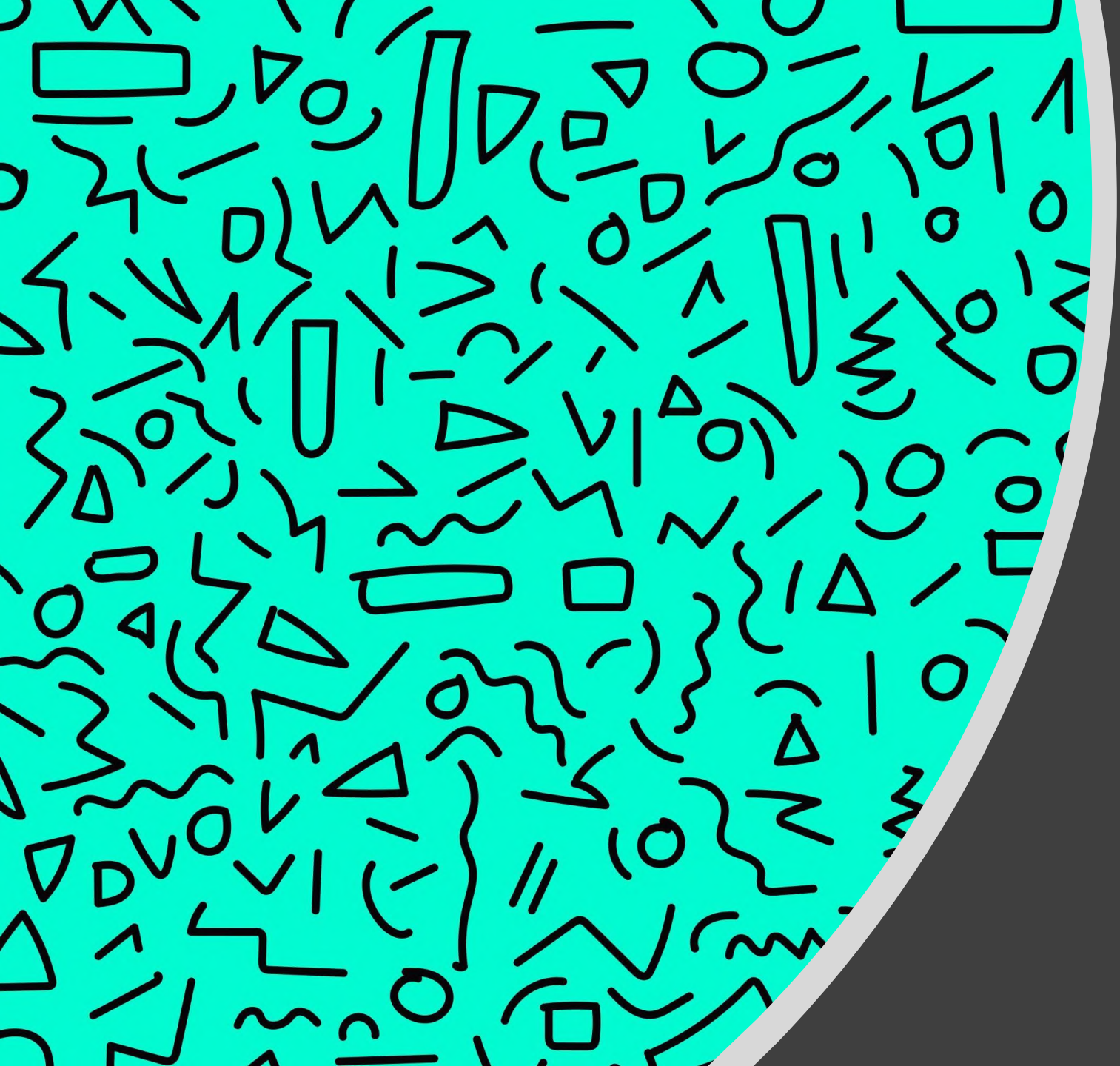
MINISTRY OF EDUCATION,
YOUTH AND SPORTS

SMART VUT, CZ.02.2.69/0.0/0.0/18_056/0013325

Microbial balances in batch and continuous fermentation



1. Calculation of balances in batch cultivation

Fermentation processess can be quantified:
1.1 by fermentation process rate

1.1 Quantification of fermentation process by fermentation process rate

We express the following:

- **the growth rate of the microorganism:**

  $$r_X = \frac{X}{dt}$$

  or **specific growth rate:**

  $$\mu = \frac{1}{X} \times \frac{dX}{dt}$$

- **the product formation rate**:

  $$r_P = \frac{dP}{dt}$$

  or **specific product formation rate:**

  $$\pi_p = \frac{1}{X} \times \frac{dP}{dt}$$

- **substrate consumption rate:**

  $$r_S = \frac{dS}{dt}$$

  or **specific substrate consumption rate:**

  $$\sigma_S = -\frac{1}{X} \times \frac{dS}{dt}$$

1.1 Quantification of fermentation process by fermentation process rate

carbon dioxide production rate:

$$r_{CO2} = \frac{dCO2}{dt}$$

or **specific carbon dioxide production rate**:

$$\pi_{CO2} = -\frac{1}{X} \times \frac{dCO2}{dt}$$

rate of the oxygen consumption in the liquid:

$$r_{O2} = q'_{O2} = \frac{dC_L}{dt}$$

where units are [g/l] or [mol/l.s]

or **specific rate of the oxygen consumption:**

$$\sigma_{O2} = -\frac{1}{X} \times \frac{dC_L}{dt}$$

where units are [s^{-1}]

Fermentation processess can be quantified:

1.2 by stochiometric relations

1.2 Quantification of fermentation process by expression of stochiometric relations

Using the schema:

$$S_i + O_2 + X_i \rightarrow X + P + CO_2 + \Delta H$$

where X_i is inoculum, S_i substrate at the start of the cultivation, X – biomass, P – product, CO_2 – carbon dioxide, ΔH – the heat produced by biochemical processes

1.3 Quantification of fermentation process by yield coefficients

The **yield coefficient for biomass from substrate**:

$$Y_{X/S} \cong -\frac{\Delta X}{\Delta S}$$

where $\Delta S = \Delta S_{assimilation\ into\ biomass} + \Delta S_{assimilated\ into\ an\ extracellular\ product} + \Delta S_{growth\ energy} + \Delta S_{maintenance\ energy}$.

In more detail the schema for growth and formation of products in aerobic environment is as follows:

The yield coefficient for product from substrate:

$$Y_{P/S} \cong -\frac{\Delta P}{\Delta S}$$

The yield coefficient for carbon dioxide from substrate:

$$Y_{CO2/S} \cong -\frac{\Delta CO2}{\Delta S}$$

1.3 Quantification of fermentation process by yield coefficients

Yield coefficient for product related to the produced biomass:

$$Y_{P/X} \cong \frac{\Delta P}{\Delta X}$$

Yield coefficient for biomass related to the used oxygen:

$$Y_{X/O2} \cong -\frac{\Delta X}{\Delta O2}$$

A ***maintenance coefficient*** **describing the specific rate of substrate uptake for cellular maintenance**:

$$m \equiv -\frac{[dS/dt]_m}{X}$$

The balance equation with maintenance coefficient for the determination of substrate consumption:

$$\left(\frac{dS}{dt}\right) = \left(\frac{dS}{dt}\right)_X + \left(\frac{dS}{dt}\right)_P + \left(\frac{dS}{dt}\right)_m$$

And therefore:

$$\frac{dS}{dt} = -\frac{1}{Y_{\frac{X}{S}}} \times \frac{dX}{dt} - \frac{1}{Y_{P/S}} \times \frac{dP}{dt} - \mathrm{m} \times \mathrm{X}$$

Categories of microbial products (models)

Categories of microbial products

1) **Growth-associated products**, which are produced during the microbial growth:

$$\pi_P = \frac{1}{X} \times \frac{dP}{dt} = Y_{P/X}\mu_g$$

Note: μ_g is different from net specific growth rate

The product formation through the associated model can be described as follows:

$$\frac{dP}{dt} = \alpha \times \frac{dX}{dt}$$

Categories of microbial products

2) **Mixed-growth-associated product**, which is formed during the slow growth as well as stationary phase:

$$\pi_P = \alpha \times \mu_g + \beta$$

The product formation through the mixed model can be described as follows:

$$\frac{dP}{dt} = \alpha \times \frac{dX}{dt} + \pi_p X$$

Categories of microbial products

3) **Non-growth-associated product**, which is formed during the stationary phase.

$$\pi_P = \beta = constant$$

The product formation through the non-associated model can be described as follows:

$$\frac{dP}{dt} = \pi_P X$$

Substrate inhibited microbial cell growth

In the case of high concentrations of substrate the microbial cell growth rate is inhibited by substrate.

The specific growth rate and kinetics parameters for noncompetitive substrate inhibition:

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

where μ_{max} is maximum specific growth rate when $S >> K_S$ (saturation constant – concentration of the rate limiting substrate when the specific rate of growth is equal to one half of the maximum), K_I concentration of inhibitor and critical parameters are:

$$S_{crit} = \sqrt{K_S \times K_I} \qquad \text{and} \qquad \mu'_{max} = \frac{\mu_{max}}{1+2\times\sqrt{K_S K_I}}$$

In the case of **competitive substrate inhibition**:

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

Product inhibited microbial cell growth

Product inhibition can be competitive or non-competitive.

Inhibition by toxic compounds

Inhibition by toxic compounds can be competitive, noncompetitive and uncompetitive

The overall productivity in batch system for the product:

$$Pp = \frac{P}{t}$$

where Pp is productivity, P – product concentration and t – time

And **the overall productivity in batch system for biomass**:

$$Pp = \frac{X}{t}$$

where Pp is productivity, X – biomass concentration and t – time

2. Calculation of balances in continuous cultivation

Continuous cultivation – calculations

The continuous cultivation is an open system with a continuous feed of nutrients and substrate. This system is designed for long-term operations. The vessel that is used as a growth container in continuous cultivation with the controlled flow rate and a constant substrate concentration is called a chemostat. In the chemostat pH, temperature and oxygen concentration are controlled.

The rate of nutrient exchange in the chemostat is expressed as a dilution rate (D):

$$D = \frac{F}{V}$$

where F is medium flow rate (F = constant) and V is culture volume (V = constant).

Continuous cultivation – calculations

Continuous cultivation can be provided also in **other types of continuous systems** such as **turbidistat**, where X is constant and D is not constant.

Next type is **nutristat**, where S is constant, and D is not constant. The last example of continuous system is **oxistat**, where C_L is constant and D is not constant.

Chemostat balance can be expressed as:

Accumulation = inflow – outflow + reaction (consumption, production)

Balance calculation for biomass:

$$\left(\frac{dX}{dt}\right)_{acc} = -D \times X + \mu \times X$$

Balance calculation for product (associated growth model):

$$\left(\frac{dP}{dt}\right)_{acc} = -D \times P + \alpha \times X$$

Continuous cultivation – calculations

Balance calculation for substrate:

$$\left(\frac{dS}{dt}\right)_{acc} = D \times S_i - D \times S - \frac{1}{Y_{\frac{X}{S}}} \times \frac{dX}{dt} - \frac{1}{Y_{\frac{P}{S}}} \times \frac{dP}{dt} - m \times X$$

After a certain cultivation time the concentration of X, S, and P will reach a steady state. This means that:

$$\frac{dX}{dt} = \frac{dS}{dt} = \frac{dP}{dt} = 0$$

The application of the stationary conditions to biomass balance can be expressed as:

$$\left(\frac{dX}{dt}\right)_{acc} = 0 = -D \times X + \mu \times X$$

therefore:

$D = \mu$

Continuous cultivation – calculations

For the washout of the culture from the continuous reactor:

$\mu = D = D_{crit}$,

μ and $\bar{X} = 0, \quad \bar{S} = S_i$

When Monod equation is applied then D_{crit} is expressed as:

$$D_{crit} = \mu_{max} \times \frac{S_i}{S_i + K_S}$$

where K_S is a saturation constant.

The productivity of the biomass in chemostat:

$$p_x = \bar{X} \times D$$

The productivity of the product in chemostat:

$$p_P = \bar{P} \times D$$


Questions?

Thank you for your attention!

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Zpráva o zavedení výuky předmětu v cizím jazyce

Příjemce	**Vysoké učení technické v Brně**
Registrační číslo projektu	**CZ.02.2.69/0.0/0.0/18_056/0013325**
Název projektu	**Studium moderní a rozvíjející se techniky VUT**

Fakulta/součást VUT v Brně:	**Fakulta chemická**
Název studijního programu:	**BSP Chemie a technologie potravin, BSP Chemie pro medicínské aplikace**
Typ studijního programu:	bakalářský
Kód studijního programu (AKVO):	B0721A210001, B0531A130015
Forma studia:	prezenční
Název předmětu:	**Bioengineering I**
Druh předmětu:	povinně volitelný
Rozsah za semestr:	12x3h
Jazyk výuky:	anglický
Způsob zakončení:	z/zk
Doporučený ročník studia:	3.
Termín zahájení výuky v cizím jazyce:	září 2021
Stručný popis/poznámka:	

The course aims to provide students with basic information in the field of Bioengineering. Students should understand the connection between bioengineering and biotechnology and should be able to solve fundamental problems and calculations related to the balancing of systems, the function of bioreactors and their necessary basic technological operations associated with their use. Another critical point in this subject is to understand the growth kinetics of microorganisms. An essential part of the course is the explanation of downstream processes. Last but not least, students will understand the kinetics of enzymatic reactions.

1. Introduction to bioengineering.
2. Thermodynamics and stoichiometry in Bioengineering
3. Bioreactors & fermenters and basic technological operations
4. Design of fermenter, types of fermenter
5. Sterilization
6. Microbial growth kinetics
7. Storage of microorganisms and media for cultivation
8. Microbial balances in batch and continuous fermentation
9. Cell growth measurement
10. Downstream processes
11. Kinetics of one-substrate enzyme-catalyzed reactions

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12. Kinetics of multisubstrate enzymatic reactions
13. Kinetics of inhibited enzymatic systems

V Brně dne 1.9.2021

prof. Ing. Martin Weiter, Ph.D.
děkan fakulty chemické

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