Bioreactor Mass & Energy Balances;
Scaling Strategies & Benefits

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1. Introduction
   Profile BioVT @ Vienna University of Technology
   Profile Exputec GmbH

2. Basics Mass & Energy Balancing
   Basic principles
   Calculation of rates and yield coefficients
   Compiling balances

3. Advanced Material Balancing Tools
   Test data consistency
   Enhance data quality
   Estimate unknowns

4. Exputec Case Studies
• Method Development
  – Upstream & Downstream Process development
  – Soft-sensors
  – PAT strategies
  – Control strategies
• Systems
  – Microbials, Fungi, Extremophiles, Cell Culture
• Strain Engineering
Exputec GmbH

- Focusing on scalability, efficiency and quality for industrial bioprocesses
- Contract Research
- Consulting
- Algorithms
Mass & Energy Balances

Basics

General material balance
Calculation of volumetric rates
Calculation of specific rates
Calculation of yield coefficients
Compiling balances
Motivation

- **Goals**
  - Bioprocess design, analysis, optimization and control

- **Challenge**
  - Complexity
  - Non-linearity
  - Framework: Time & Cost

→ Elemental and energy balancing is primary tool for bioprocess analysis
Elemental and energy balancing is applied during the whole bioprocess development and product lifecycle.
General material balance

- Principle
  - Conservation of mass, charge, energy
- Basic bioprocess analysis tool
  - Total mass
  - Elements (C, N, O, H, S etc.)
  - Energy (e.g. enthalpies)
Simple example from chemical engineering

- **Simple mass balancing example**
  - 1 inlet stream
  - 3 outlet streams
  - outlet concentrations [%] and inlet stream [g/h] is measured

- Outlet streams (F1, F2, F3) should be calculated
Simple example from chemical engineering

- **Approach**
  - define envelope
  - Set up equations
  - compute unknowns

- **Balances used**
  - Total mass balance, species 1 and species 2 balance

- **Outlet streams can be calculated**

**Equations**

\[
\begin{align*}
x_1 + x_2 + x_3 &= 10 \\
0.04 \times x_1 + 0.54 \times x_2 + 0.26 \times x_3 &= 10 \times 0.2 \\
0.93 \times x_1 + 0.24 \times x_2 + 0.00 \times x_3 &= 10 \times 0.6
\end{align*}
\]

\[
x_1 = 5.8; \ x_2 = 2.4; \ x_3 = 1.7
\]
Mass balancing of bioprocesses
- calculation of volumetric rates, specific rates and yield coefficients
- estimation of unknown non-measured variables
- consistency checking of measurements
What kind of reaction takes place?

Stoichiometry

- Very complex!
- Alternative: Black box approach
  - Cell considered as catalyst
- Concept of C-molarity
  - Every species divided through the number of C-atoms

\[ \text{INPUT} \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \quad \qut...
Volumetric rates

- **Volumetric rates** [mol/(L*h)]
  - How much of species \( i \) is processed by liter and hour
  - Examples: OUR, CER, MER, rx

- **General approach**
  - Take general material balance
  - Solve for rate (\( r \))

Example: Batch Process, Biomass

\[
\dot{V}_{in} * c_{in} - \dot{V}_{out} * c_{out} + V_R * r_i = V_R * \frac{\partial c_i}{\partial t} + c_i * \frac{\partial V_R}{\partial t}
\]
Volumetric rates

- **Volumetric rates** [mol/(L*h)]
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Example: Batch Process, Biomass

\[
\dot{V}_{in} \cdot c_{in} - \dot{V}_{out} \cdot c_{out} + V_{R} \cdot r_{i} = V_{R} \cdot \frac{\partial c_{i}}{\partial t} + c_{i} \cdot \frac{\partial V_{R}}{\partial t}
\]

\[
dV/dt = 0
\]

\[
\frac{dx}{dt} = rX
\]
Specific Rates

- **Calculation**
  - Divide volumetric rates through amount of catalyst (biomass)

- **Specific rates [g/cell/h]**
  - How much of species is converted by catalyst (biomass) and hour
  - Examples: $\mu$, $qs$, $qM$
  - Link to physiology!

\[
\frac{r_i}{x} = q_i
\]
Yield Coefficients

- **Calculation**
  - Divide rate/ rate or specific rate/ specific rate

- **Yield coefficients [g/g]**
  - Examples: Biomass yield coefficient (Yx/s), Respiratory quotient (RQ)
  - Physiological interpretation!

\[ Y_{i/j} = \frac{r_i}{r_j} = \frac{q_i}{q_j} \]
Volumetric rates vs. specific rates and yield coefficients

- **Basic bioprocess descriptors**
  - Volumetric rates
    - carry information on total metabolic activity
  - Specific rates
    - carry information on cell physiology
  - Yield coefficients
    - carry information on cellular flux distributions

→ Calculate for every process

\[ Yx/s = rx/rs \]
Compiling balances - Start to trust your rates

- **Elemental Balances**
  - C-Balance
  - N-Balance
  - Degree of Reduction balance

- **Energy Balances**
  - Enthalpies of species

> Can I trust my data? Stoichiometry right?
Practice of calculating time-resolved rates, yield coefficients & balances

- Suggested procedure
  - 1) Align Data
  - 2) Calculate volumetric rates
    \[
    \dot{V}_{in} \cdot c_{in} - \dot{V}_{out} \cdot c_{out} + V_R \cdot r_i = V_R \cdot \frac{\partial c_i}{\partial t} + c_i \cdot \frac{\partial V_R}{\partial t}
    \]
  - 3) Calculate specific rates, yields and compile balances
    \[
    \frac{r_i}{x} = q_i \quad Y_{i/j} = \frac{r_i}{r_j} = \frac{q_i}{q_j}
    \]

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Variables

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Constants

- \( V_m = 22.4 \)
- \( \text{DoR}_S = 4 \)
- \( \text{DoR}_{O2} = -4 \)

Rates/ Yields/Balances

<table>
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<tr>
<th>Time [h]</th>
<th>rs [Cmol/L/h]]</th>
<th>qs [Cmol/(gh)]</th>
<th>Carbon balance</th>
<th>DoR balance [g/l]</th>
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<td>0.632</td>
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Example: timely resolved rates for an *E. coli* fermentation

Aquired raw data

Fermentation parameters: induction temperature: 20°C, k=0 (linear feed).

**Figure 85:** Fermentation at 20°C applying a linear feed (k=0). Left: intracellular protein concentrations (orange), intracellular activity (purple) and biomass concentrations (green). Right: extracellular protein- (orange) and biomass (green) concentrations. X-axis: induction time.

**Figure 86:** Fermentation at 20°C applying a linear feed (k=0). Left: off-gas measurements of CO₂ (green), oxygen (blue) as well as gas inlet flows of oxygen (purple) and air (orange). Right: signals recorded from the feed balance (orange) and base balance (purple). Time point of induction is indicated by a vertical bar. X-axis: process time.
Example: timely resolved rates for an *E. coli* fermentation

**Volumetric rates, specific rates and balances**

Fermentation parameters: induction temperature: 20°C, k=0 (linear feed).

**Figure S7**: Fermentation at 20°C applying a linear feed (k=0). Left: volumetric growth rate $\mu$ (blue), volumetric substrate uptake rate $r_S$ (red), protein release rate $r_p$ (purple) and volumetric cell lysis rate (red). Right: specific growth rate $\mu$ (blue), specific substrate uptake rate $q_S$ (orange), specific protein release rate $q_{Pr}$ (purple) and specific cell lysis rate $q_{Cell}$ (red). X-axis: induction time.

**Figure S8**: Fermentation at 20°C applying a linear feed (k=0). Left: biomass (blue), CO$_2$ (orange), extracellular protein (green) as well as ammonia (purple) yield. Right: carbon (blue), degree of reduction (orange) as well as nitrogen (green) balance.
Remark 1) Gas flow quantification via MFCs

- **MFCs**
  - **Mass flow controller**
    - Calibrated for defined gases
    - Not calibrated for complex mixtures of gases

- **Outflow**
  - $F_{in} = F_{out}$? No!
    - Reactions $rO_2$, $rCO_2$
    - $H_2O$ vapour

\[ q = F \times C_p \times \Delta T \]
Remark 2) Accumulation of gaseous species

Accumulation term

- Neglectable?
  - Gas holdup
  - Temperature change?
  - pH change?

Corrections

- T-dependency
  - Henry’s Law: $k_{Hf}(T)$

- Total carbonate
  - Correct using carbonate mass balance and acid equilibrium
Remark 3) Scaling Issues

- Reactor In-homogeinities
  - Organism can produce overflow metabolites in compartment 1, overflow metabolites are consumed in compartment 2
  - → Yield coefficients change as a function of scale
Summary Basics Material Balancing

- General Material Balance
- Stoichiometry
- Volumetric rates
- Specific rates
- Yield Coefficients
- Balances

\[
\dot{V}_{in} \cdot c_{in} - \dot{V}_{out} \cdot c_{out} + V_R \cdot r_i = V_R \cdot \frac{\partial c_i}{\partial t} + c_i \cdot \frac{\partial V_R}{\partial t}
\]

\[
CH_mO_i + b \cdot NH_3 + c \cdot O_2 = a \cdot CH_pO_nN_q + dCH_rO_sN_t + eH_2O + fCO_2 + \text{energy}
\]

\[
\frac{r_i}{x} = q_i
\]

\[
\frac{Y_{i/j}}{r_j} = \frac{q_i}{q_j}
\]

\[
\frac{rs \cdot 1 + r_{O_2}}{r_{CO_2} \cdot 1 + r_x \cdot 1 + r_p \cdot 1} = 1
\]
Mass & Energy Balances

Advanced tools

Testing for data quality
Enhance data quality
Estimate unknown rates
(1) Rates Calculability/ Balancability/ Redundancy

- **Classification of rates**
  - Calculable
  - Non-calculable
  - Balancable
  - Non-balanceable

- **Redundancy**
  - Two or more of the measured conversion rates are balancable

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(1) Test for data quality/ reliability

- Can I trust my data?
  - Outliers
  - Sensor drifts
  - Instrument failure

- Material balancing
  - Can error on balances be explained by measurement noise?

→ Compute statistical test value to test for data quality

(2) Enhance data quality

- If no gross errors
  - Use reconciliation to enhance data quality
    - adapt rates so that all balances close simultaneously
    - Noise-eliminated rate
    - Approach: weighted least squares

\[
W_b = W + \delta
\]

(2) Enhance Data Quality in Real-Time

- Data reconciliation
  - can be performed in real-time
  - Elimination of noise
  - No smoothing!
  - Checks for data consistency in real-time

→ Higher signal to noise ratio
→ More information from your data
→ Gain trust in your data through statistical test value (quality criteria for your data)
(3) Estimate unknown reaction rates

- **Estimation**
  - Check Rank (Redundancy)
  - Reconcile measured rates
  - Estimate rates
  - Check estimation quality using statistical test value

- **Reliable estimation of unknown rate**

---

(4) From estimated rate to estimated concentration

- **Soft sensing**
  - Numerical cumulation of estimated rate (e.g. biomass formation rate)
  - Estimation of volume following a mass balancing approach

→ Reliable estimation of unknown process variable

Summary Advanced Tools

- **Trust in data** consistency using a statistical test
- **Less noise** on data through reconciliation
- **Estimate unknown rates**
- **Estimate unknown concentrations**
Mass & Energy Balances

Case studies
Material balancing assisted Multivariate Data Analysis
Platform Control Algorithms
Reconciliation of Gas Rates
(1) Using mass balancing in combination with Multivariate Data Analysis (MVDA)

- MVDA
  - Tool used to analyze large data sets (MLR, PCA, ParaFac, Tucker 2, 3 …)
  - Factors → Responses
  - Which factors to use?
    - Concentrations?
    - Volumetric rates?
    - Specific rates?

→ Run MVDA on specific rates and yields instead of raw data

RAW Data e.g. [CO2]

Information e.g. CER, qCER, μ

(1) Using mass balancing in combination with Multivariate Data Analysis (MVDA)

- **Challenge**
  - Inconsistent data sets
    - Measurements missing
    - Sensor drifts, errors?

- **Solution**
  - 1) Test data consistency, remove data that is not consistent
  - 2) Reconcile measurements
  - 3) Estimate non-measured rates and yields

→ Provision of reconciled specific rates and yield coefficients as input for MVDA
(1) Using mass balancing in combination with Multivariate Data Analysis (MVDA)

- Example recombinant *E. coli* bioprocess
  - Processing of data (concentrations, flows) into specific rates and yield coefficients ($\mu$, $qs$)
  - Principal Component Analysis

$\rightarrow$ Root cause identified, variables impacting on process performance can be interpreted
(2) Development of a platform bioprocess control algorithm

- **Goal:** closed-loop control of the specific growth rate
  - Fed-batch process
  - primary process variable in respect to product formation
  - Two hosts, many strains, many products, many processes
  - Desired: One algorithm that fits them all

→ Develop one platform control algorithm to control the specific growth rate in microbial processes
(2) Development of a platform bioprocess control algorithm

- **Approach**
  - **Toolset**
    - Data Reconciliation, Rate Estimation, Rate Cumulation
    - Data Base for Host stoichiometry
    - On-line detection of physiological state using a statistical test
    - Control Algorithm

→ Advanced balancing tools and on-line statistical decision making for the development of a generic control algorithm
(2) Development of a platform bioprocess control algorithm

- In process performance
  - Implementation: Compiled to C, runs in real time on Lucullus PIMS
    - No strain specific knowledge used
    - Specific growth rate controlled
    - Specific substrate uptake rate of inducing substrate controlled dynamically

→ Robust control of the specific growth rate in recombinant *E. coli* bioprocesses
(3) Estimate methane evolution rate in a methanogenesis process

- **Process**
  - Conversion of H2, CO and CO2 to CH4

- **Goal: Reliable on-line estimation of product formation**
  - Use of MFCs requires Cp correction through measurement (H2, CH4, CO2 (different Cps))
  - On-line estimation of reaction rates and consistency check

→ Estimate product formation rates in real-time
Take home messages Mass & Energy Balancing

- Balancing is key bioprocess analysis tool
  - Volumetric & specific rates, yield coefficients
  - Compiling balances (C, DoR, N, S, etc.)
- Sound balancing is a balancing act
  - Are simplifications justified?
  - Sensor reaction time?
  - Changes in pH, T that effects gas solubility?
- Scaling
  - Yield coefficients can change due to reactor in-homogeneity
Take home messages Advanced tools

- Balancing tools do not stop with compiling balances!
  - Use statistical test to detect sensor drifts, miscalibration and gain trust in your data
  - Use reconciliation and rate estimation to compute unknown fluxes in real time
  - Obtain a statistical test value for your estimation quality in real time!
- Use a powerful toolset of advanced balancing tools for bioprocess design, analysis and control
  - Multivariate Data Analysis
  - Observer (Particle Filter, Kalman etc)
  - Control algorithms
Take home messages Case Studies

- Use a powerful toolset of advanced balancing tools for bioprocess design, analysis and control
  - Multivariate Data Analysis
    - Only use data that is trustworthy
    - Quality criteria for your data
  - Genetic platform control strategies
  - Applicable for white & red biotechnology
EXPUTEC

*Design to Improve*

*Bioprocess solutions*

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Backup Slides
\[ h = \varepsilon^T \Phi^{-1} \varepsilon \]

\[ \delta = \Psi \varepsilon^T \Phi^{-1} \varepsilon \]

\[ EX = E_m X_m + E_c X_c = 0 \]

\[ X_c = E_c^{-1} E_m X_b \]