Mass & Elemental Balancing for Bioprocess Data Analysis

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23. 10. 2015
Introducing Exputec

- Exputec, based in Vienna, Austria, is a technology-driven company, offering solutions for efficient **process development** and **manufacturing**.

- Exputec provides **consulting services** and **software solutions** to clients in the biopharma, industrial biotechnology, sustainable biotechnology, and chemical sectors.
Challenges solved by Exputec data science

Screening
- faster process development
- higher productivity
- guide design decisions

Process
- prevent and identify scale-up effects
- efficiently characterize & develop risk assessments

Piloting
- prevent failed batches
- continuous improvement
- technology transfer

Manufacturing
How do we do that?

- **Clear and proven methods** for data analysis & mechanistic modelling
- **Efficient use of multivariate and mechanistic data science methods**

→ Bioprocess development and manufacturing challenges can be solved by **process data science**
→ We provide these solutions to the customers in the form of **data science consulting** and **software**
Advanced upstream process analysis & scale-down model

- Mass and energy balancing basics
- Implementation and pitfalls
- Applications: Scale-up and process control
- Take home messages
Mass and Energy Balancing

- **Motivation**
  - Bioprocess design, optimization, economic evaluation, troubleshooting etc.

- **Process information**
  - Reaction rates, step yields, C-yields, metabolic fluxes, growth rates

→ Information on fluxes, reaction rates and yields are necessary for process data analysis and optimization
→ Therefore, elemental and energy balancing is primary information mining tool for bioprocess analysis
Mass and Energy Balancing

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Mass and energy balancing

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

→ Basic principle: Conservation of mass and energy
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**
  - Step 1: define envelope
  - Step 2: Set up
  - Step 3: Calculate unknowns
- **Balances used**
  - Total mass balance, species 1 and species 2 balance
- **Outlet streams can be calculated**

**Equations**

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

\[
x_1 = 5.8; \ x_2 = 2.4; \ x_3 = 1.7
\]
Same story for bioprocesses

- Mass balancing of bioprocesses
  - calculation of volumetric rates, specific rates and yield coefficients
  - estimation of unknown non-measured variables
  - consistency checking of measurements (C-balance, DoR-balance, N-balance)

\[
\dot{V}_{\text{in}} * c_{\text{in}} = \dot{V}_{\text{out}} * c_{\text{out}} + V_R * r_i
\]

\[
= V_R \frac{\partial c_i}{\partial t} + c_i \frac{\partial V_R}{\partial t}
\]
Information mining via fermentation balancing

- Volumetric reaction rates
  - Basis for process design (e.g. OTR,max)
- Specific reaction rates
  - Directly interlinked with productivity and product quality
  - (e.g. specific growth rate)
- Yield coefficients
  - Direct metabolic interpretation, insights in intracellular flux distributions

Input

\[ CH_m O_l + b \times NH_3 + c \times O_2 = \]

\[ \alpha CH_p O_n N_q + d CH_r O_s N_t + e H_2 O + f CO_2 + \text{energy} \]
Calculation of volumetric reaction rates

- **Volumetric rates** \([\text{mol}/(\text{L}*\text{h})]\)
  - 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

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

\[
\frac{dx}{dt} = r \cdot x
\]

Example: Batch Process, Biomass

\(\text{IN} = 0\) \hspace{1cm} \(\text{OUT} = 0\) \hspace{1cm} \(\text{Volum. rate}\)
Calculation of 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

\[ \frac{r_i}{x} = q_i \]
Applications of specific reaction rates

- Process analysis
  - The specific growth rate strongly impacts on the specific productivity and product quality
- Technology Transfer
  - Assess scale- and technology transfer criterion
  - Scale-up criterion
    - Physiological similarity

Highly diagnostic information to analyze and optimize bioprocesses
Calculation of yield coefficients

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

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

\[ Y_{i/j} = \frac{r_i}{r_j} = \frac{q_i}{q_j} \]
Interpretation of biomass yield coefficient

- Fraction of biomass formed per substrate consumed
  - Changes dynamically in a bioprocess
    - Indicator for metabolic load in recombinant processes
  - Can change during scale-Up
    - Biomass yield coefficient changes during scale-up due to large scale inhomogenities

Highly diagnostic information for the analysis of bioprocess scale-ups and recombinant systems

Substrate gradient formation in the large-scale bioreactor lowers cell yield and increases by-product formation
F Bylund, E Collet, SO Enfors, G Larsson - Bioproc
Advanced upstream process analysis & scale-down model

- Mass and energy balancing basics

- Implementation and pitfalls

- Applications: Scale-Up and Process Control

- Take home messages
Common pitfalls in mass balancing (1)

- **Mass flow controllers**
  - **Mass flow controller**
    - Calibrated for defined gases
    - Not calibrated for complex mixtures of gases
  - **Outflow**
    - \( \text{Fin} = \text{Fout} \)? No!
      - Reactions \( r\text{O}_2, r\text{CO}_2 \)
      - \( \text{H}_2\text{O} \) vapour

\[ q = F \times C_p \times \Delta T \]

Highly diagnostic information for the analysis of bioprocess scale-ups and recombinant systems
Common pitfalls in mass balancing (2)

- Accumulation of gaseous species
  - Neglectable?
    - Gas holdup
    - Temperature change?
    - pH change?

- Corrections
  - T-dependency
    - Henry’s Law: $k_H f(T)$
  - Total carbonate
    - Correct using carbonate mass balance and acid equilibrium
Common pitfalls in mass balancing (3)

- **Representative sampling**
  - Is the sample and are the analytes changing after they were taken from the bioreactor?

- **Considerations**
  - Fast (immediate) separation of biomass and substrates can only be achieved by rapid sampling and filtration devices
  - Fermentation matrix contains extracellular proteins, DNA, etc. Should be considered when choosing the right enzymatic assay or HPLC method
Practice of calculating time-resolved rates, yield coefficients & balances

1) Align data
2) Calculate volumetric rates

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

3) Calculate specific rates, yields and compile balances, test quality of data

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<table>
<thead>
<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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<tr>
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<td>0.005</td>
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<td>0.632</td>
<td>0.0051</td>
<td>1.05</td>
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Variables

<table>
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<tr>
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<th>Vin</th>
<th>Vout</th>
<th>X</th>
<th>Prod</th>
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</tbody>
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Constants

\( V_m = 22.4 \)
\( \text{DoR}_S = 4 \)
\( \text{DoR}_{O2} = -4 \)
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Technology Transfer & Scale-Up

- Process was transferred to another site, process step was changed or process scale-up was performed
- Can the processes be considered similar?

- Can process A be considered similar to process B? What changed during scale-up?

→ Exputec assesses the similarity of processes based on multivariate methods
Scale-up analysis

- **Identify**
  - Identification of scale-dependent deviations
- **Understand**
  - Identify root causes
- **Improve**
  - Identify design patterns to avoid scale dependent deviations

→ Improve robustness and predictability of scale-up
Scale-Up Analysis - Methods

- **Data Alignment & Preprocessing**
  - Align and ensure data quality
  - C,N, DoR balance to gain trust in data

- **Information Mining**
  - Control qualities of variables, specific rates, yield coefficients, dimensionless numbers

- **Multivariate Methods**
  - To allocate differences and interdependencies
Scale-up analysis – Identify and Understand

- Clear identification which parameters are changing during scale-up
  - Temperature, dissolved oxygen: Analysis of set-points and control quality
  - Analysis of scaling of feeding regimes
  - Specific uptake rates and yield coefficients

→ Comprehensive results
→ Which variables change during scale-up?
→ Knowledge on expected/unexpected changes
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  - Scale-up
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- Process Development
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- Piloting
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- Manufacturing
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
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
Development of a platform bioprocess control algorithm

- In process performance
  - Implementation on PLS systems
    - 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
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Take home messages mass & energy balancing

- Balancing is key bioprocess analysis tool
  - Volumetric & specific rates, yield coefficients
  - Compiling balances (C, DoR, N, S, etc.)

- Physiological interpretation of specific rates and yield coefficients
  - Parameters have physiological meaning and can indicate

- Pitfalls
  - Are simplifications justified?
  - Changes in pH, T that effects gas solubility?
Take home messages case studies

- 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
  - Control algorithms
Thank you for your attention!