Basic Operations with Bioreactors – Process Control

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Agenda

• What typically includes the tasks of a bioprocess?
• Bioprocess automation: A few definitions
• Control strategy of bioprocesses
• Process analytical technology (PAT)
• Summary
Basic Operations with Bioreactors – Process Control
General sequence of a bioprocess

UPSTREAM PROCESSING → FERMENTATION → DOWNSTREAM PROCESSING

- **Sterilization** of equipment
- Cleaning (CIP)
- Preparation of medium
- Calibration
- Inoculum preparation

- Biomass and product formation

- Preparation of biomass
- Product isolation and purification

**Note:** Generally « fermentation » is considered being part of upstream (USP) processing.
Process operation: empirical „more an art than science“

Mass balance

Substrate + O$_2$ $\rightarrow$ Biomass + Product + H$_2$O + CO$_2$ + heat
Make life easier: Process automation

But first a few definitions…

- **Process**: Integrated system by which the transformation and/or transport of materials, energy and/or information is accomplished.

- **Process control**: Automatic control of a process, in which a computer system is used to regulate the usually continuous operations or processes.

- **Process monitoring**: Acquisition of key variables.

- **Automation**: Sequence of operations running without the interaction of man but following adapted algorithms. Advanced level of automation is robotics.
Fermentation process

Main characteristics:
• complex
• highly interactive
• parallel
• non linear

Role of automation

• Control of state variables
  Temperature, pH, pO₂, rpm, conductivity, pressure, etc.
• Control of procedures
  * sampling
  * feed (substrate, inducer, precursor,...)
  * harvest
• Documentation

Impact of automation is low with respect to acquisition of key variables.
Process control needs for different fermentation strategies

Batch cultivation

Monitoring and control of state variables:
- pH
- pO₂ (agitation and air flow)
- Temperature
Process control in different fermentation strategies

Fed-batch cultivation-growth control by exponential substrate feed

- Monitoring of state variables (pH, pO₂, temperature, rpm...)
- Substrate feed to maintain constant growth rate

Substrate feed according to biomass growth:

$$x = x_0 e^{\mu t}$$

$$S = \frac{1}{Y} \frac{x}{x_0} e^{\mu t}$$
Bioprocess control

For the use of microorganisms or cells as producers of products, it is necessary to analyse and control the biological, chemical and physical surroundings continuously.

Sensors and systems for the monitoring, control and regulation of complex processes = process control systems (process computer).
Process control and automation

Goal: optimal utilisation of biological systems

- Biosynthesis
- Transformation of a product
- Degradation

Consider: Impact of process control on bioprocess

Efficiency (yield) of a bioprocess is determined by

- Performance of the cell factory
- Environmental parameters

=> Main tasks:

- Maintenance of process conditions by monitoring and control
- Documentation
Reasons for increasing need for automation and use of computers in bioprocessing

There is a need for accelerated process development and increased efficiency and **economics** of production processes!

**Process level**
- Process regimes became far more complex and sophisticated e.g. (batch ➔ fed batch)
- Continuous increase of variables to be monitored
- Increasing complexity of variables

**Level of quality management**
- Demands related to quality management increased dramatically (hidden plant, makes approx. 35% (up to 70%) of costs)
- Increased demands on documentation (GMP)
Bioprocess automation

accepted standard of M&C:
Measure/Monitor

Control (open loop)
MESSEN

Measure/Monitor

STEUERN

Control (open loop)

REGELN

PID regulation

\[ u = K_p \cdot e \]

\[ u = K_p \left( e + T_d \frac{de}{dt} \right) \]

\[ u = K_p \left( e + T_d \frac{de}{dt} + \frac{1}{T_i} \int_0^t e(\tau) d\tau \right) \]
Feed-back control loop (closed loop)

...more problematic are biological signals
• **STATE VARIABLES (MONITORING)**
  • on line: pH, pO$_2$, temperature, rpm, biomass etc.
  • off line (biomass, product, residual substrate, etc.)

• **CONTROL VARIABLES:**
  feed rate (exponential feed), temperature, rpm etc.

• **QUALITY VARIABLES:**
  • rates (qP, OUR, CER, RQ) (material balancing)
  • amount and physiological activity of biomass
  • key metabolites
Bioprocess automation: What are the limitations?
Classical control scheme of fermentation process
single, separated loops without networking of variables
Reflux cooler

Ports of probes

Signal transmitter and controller

Communication with PC (visualization and configuration)

Controller

Laboratory scale bioreactor
**Definitions**

**on-line - off-line**

**on-line:** measurement can be registered directly. e.g. pH measurement

**off-line:** sample taken and analysed extern / preparation of samples ⇒ delay

**in-situ - ex-situ**

**in-situ:** sensor inside bioreactor

**ex-situ:** sample transferred to an external device for analysis

**Wish:** on-line, in-situ
Classical control scheme of fermentation process
single, separated loops without networking of variables

\[
\text{Signalconverter} \\
\text{amplifier} \\
P_{\text{H}} \text{, } P_{\text{O}_2} \\
\text{Temp.}, \text{stirrer,} \\
\text{weight (volume),} \\
\text{air flow}
\]

\[
P_{\text{H}} \text{ IC} \\
P_{\text{O}_2} \text{ IC} \\
\text{Temperature} \text{ IC}
\]

\[
\text{Laute} \\
\text{weight RIC}
\]

\[
\text{controller} \\
\text{Signalconverter} \\
\text{amplifier} \\
\text{pH, } P_{\text{O}_2}, \text{Temp., stirrer,} \\
\text{weight (volume),} \\
\text{air flow}
\]

\[
\text{R...record} \\
\text{I....indicate} \\
\text{C..control}
\]

\[
\text{Cooling water} \\
\text{Hot water} \\
\text{steam} \\
\text{weight RIC}
\]

\[
\text{Media stream} \\
\text{Measurement signals} \\
\text{Control}
\]
Fermentation process monitoring and control
concept for hierarchical bioprocess automation

analytical interfaces:
- CPU, RAM, ...
- off-line analyses

manual transport

sampling interfaces:
- filters
- pumps
- valves

physical interfaces:
- amplifiers
- converters
- power supply

media

conditioning

intelligent analytical subsystems:
- CPU, RAM, disk
- FIA, FFF, GC, HPLC, MS, ......

hard-wired

front end process control:
- CPU, RAM, ...
- control loops, sequences, alarms, security, operating, visualization, software sensors
Understanding the bioprocess in detail!

Process parameters

- heater (temperature)
- stirrer (rpm)
- substrate feed (kg, kg h^{-1})
- educt feed (kg, kg h^{-1})
- airflow (L h^{-1})
- acid base (kg, kg h^{-1})
- antifoam (kg, kg h^{-1})

Process model

State variables

online:
- gas analysis (O_2, CO_2, CO_2_{mt}, OUR, CPR, RQ)
- temperature, pH, pO_2

offline:
- biomass (kg, kg L^{-1})
- substrate (kg, kg L^{-1})
- product (kg, kg L^{-1})
- educt (kg, kg L^{-1})
- optical density / colour

Target variables

- growth rate (h^{-1}, kg L^{-1} h^{-1})
- production rate (kg L^{-1} h^{-1})
- yield Y_{k/s}, Y_{p/e} (kg kg^{-1})
- feed rates (kg kg^{-1} h^{-1}, kg L^{-1} h^{-1})
- production rate (kg kg^{-1} h^{-1}, kg L^{-1} h^{-1})

Each bioprocess has its own specialty!
HES-SO is implementing the most modern automation within the project SYNPOL (www.synpol.org)
Bioreactors Layout

**Typhoon_1**
Infors Labfors 13l with CIP unit

**Fume hoods**
With Gas Detectors (Safety: H₂, CO, CO₂)

**Typhoon_2**
Infors Labfors 3.6l

**Infors Iris 6.0**
HMI

**Infors Touchfors**
Touch panel

**Hiden**
Mass Spectrometer

A. Vaccari, M. Sequeira
System Layout

Sample from the environment or calibration gas

Process Control Unit (PCU) + Liquid Handling Automation & Datalogging

Bioreactor 1

Bioreactor 2

Liquid Handling (LH)

Flowcytometer (FC)

Gas flow
Liquid flow
Measures
Control

Gas analyzer (MS)

PC2

PC1

PC3

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Mass Spectrometer

We define:

9 measurement ports:

- Calibration_gas_1
- Calibration_gas_2
- Calibration_gas_3
- Calibration_gas_4
- Air
- Typhoon_1_IN
- Typhoon_1_OUT
- Typhoon_2_IN
- Typhoon_2_OUT

Each Port has 8 gas components:

- H₂, CO, CO₂, N₂, H₂O, O₂, Ar, H₂S

= 72 gas measurements

QGA Pro software is made with LabVIEW → Communication of data is easy! (shared variables)
BD Accuri Flow Cytometer

The built-in remote function gives us only the total cell count! → So we developed a complete new LabVIEW application that reads all fluorescence intensities and light scattering signals from the produced FCS files (gating and calculation are included):

A. Vaccari, M. Sequeira
Automatic Probe Sampling for the Flow Cytometer

Temperature controlled box (20-60 °C)

3 Hamilton valves / 2 syringes / 2 magnetic stirrers

2 home-made mixing chambers (dilution / staining)

National Instruments CRIO PLC (LabVIEW) for automation of sampling sequence


A. Vaccari, M. Sequeira
So what can we do with this new process technology?
Process Parameters (PPs)
- Temperature
- Stirrer Speed
- pH
- Dissolved Oxygen
- Air Flow
- Pressure
- Feedrate
- Nutrient Concentrations
- Biomass Concentration

How to elaborate this highly complex relationship???

Quality Attributes (QAs)
- Protein folding
- Glycosylation pattern
- Stability
- Impurities
- Batch-to-batch variability
- Ease of further processing (Downstream)

...
Critical Business Attributes are NOT an integral part of PAT and QbD! However, they are important economic driving forces.

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PAT compliant generation of Process Understanding, and its transfer from Process Development to Scale-up and Production . . .

. . . reflects an integral approach for transformation of raw data into process understanding and its use in process control. The concept of determination of specific rates allows a scale-independent transfer of a developed process into industrial manufacturing.

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Bioprocess design is a challenging task, because the bioprocess needs to be understood properly.

Upstream processing is also engineering and can significantly affect outcome of bioprocesses.

Besides GMP and other norms, new regulations at FDA require tight control and monitoring of bioprocess (process analytical technology, PAT).

Scale-up of bioprocesses can be simplified by PAT.
Why not coming to Switzerland next spring?

We organize a Workshop ‘Labview for Biotechnologists‘!

**LabVIEW** (Laboratory Virtual Instrument Engineering Workbench) is a graphical programming language from National Instruments.

**Date**: 4-5-6<sup>th</sup> June 2014  
**Location**: HES-SO Valais, Sion (CH)  
**Pre-requisites**: standard informatics user
Thank you for your attention!

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