Bioreactor process control for bacterial syngas fermentations:

1. Measurement tools specific to syngas fermentations

Stéphanie Follonier
SYNPOL’s 4th Annual Course
“Biopolymers from bacterial fermentation of syngas”

Team (fermentation part)

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Field of Expertise

- Batch, fed-batch, chemostat
- Bioreactors 0.5 to 300 L
- Medium design
- Bioprocess design
- Flow cytometry
- Process Analytical Technology
Bioreactor process control

- **Goal**
  - Ensure the success of a bioprocess
  - Adapt parameters in case of deviation
  - Provide comprehensive information for understanding and optimizing the process
  - Provide information for assessing the quality of a process (Quality by Design)

- **Traditional measurements**
  - On-line: pH, pO₂, T, agitation
  - At-line: e.g. glucose
  - Off-line: e.g. OD₆₀₀, products

Most of these measurements are related to the macroscopic features of the culture broth!
Syngas fermentations for the production of PHA

Syngas (produced from complex organic wastes)

- Safety
- Gas composition (MFC)
- Gas consumption/production (MS)
- Anaerobiosis (redox potential)

Bioprocess

- PHA production (FCM)
- Cell growth (FCM)

Biopolymers (PHA) or precursors
Control and monitoring of the syngas composition

QIC Biostream (Hiden) on-line mass spectrometer

- Powerful: can be used simultaneously for up to 48 gas streams
- Settings and calibration critical to get accurate data!
- The settings have to be chosen depending on the process (e.g. low $O_2$ concentration,...)
Control and monitoring of the syngas composition

Fragmentation pattern of the analyzed gases

<table>
<thead>
<tr>
<th>Fragment mass</th>
<th>Relative intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>- element library</td>
<td>- measurement with pure gas</td>
</tr>
</tbody>
</table>

Complex calculations required!
Control and monitoring of the syngas composition

Calibration procedure

1. Background with argon
2. Calibration with ambient air
   - Cal H₂O
   - (Cal O₂)
   - (Cal Ar)
3. Calibration with syngas
   - Cal CO
   - Cal H₂
   - Cal CO₂
   - Cal N₂

• Calibration with > 1 mixtures
  => The values have to be normalized with a common gas (here N₂)
• The calibration gases should have a composition similar to the gas used in the process

Buck equation (1981)

\[ p_{\text{H}_2\text{O}} [\text{mbar}] = (1.0007 + 3.46 \cdot 10^{-6} P) \cdot 6.1121 \exp \left( \frac{17.502 \cdot T}{240.97 + T} \right) \]

(P in mbar and T in °C)
Control and monitoring of the syngas composition

Verification test

[Graph showing syngas mixes and gas concentrations over time]

[Bar chart showing gas concentrations for different gas mixes]
Control and monitoring of the syngas composition

Example: 3-step bioprocess for the production of PHA with *R. rubrum*

- Syngas used as C source during the 3\textsuperscript{rd} step
- Measurement of exhaust gas concentrations by mass spectrometry
- Calculation of gas variation and total amount of gas produced/consumed

\[
\dot{n}_{CO}[\text{mol min}^{-1}] = \frac{F_G}{V_m} \left( x_{CO,\text{out}} \frac{x_{N_2,\text{in}}}{x_{N_2,\text{out}}} - x_{CO,\text{in}} \right)
\]

\[
\dot{n}_{H_2}[\text{mol min}^{-1}] = \frac{F_G}{V_m} \left( x_{H_2,\text{out}} \frac{x_{N_2,\text{in}}}{x_{N_2,\text{out}}} - x_{H_2,\text{in}} \right)
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\dot{n}_{CO_2}[\text{mol min}^{-1}] = \frac{F_G}{V_m} \left( x_{CO_2,\text{out}} \frac{x_{N_2,\text{in}}}{x_{N_2,\text{out}}} - x_{CO_2,\text{in}} \right)
\]

\[F_G = \text{total gas flow rate [NL mol}^{-1}\text{]}\]
\[V_m = \text{molar volume at normal conditions[L mol}^{-1}\text{]}\]

- Ratio CO\textsubscript{2}/CO and H\textsubscript{2}/CO (\(~\text{RQ for aerobic cultivations})
- Carbon balance
The redox potential (ORP)

Production of photosynthetic membranes in *R. rubrum*

Microaerobic growth in the dark on fructose and succinate with ORP ranging from 0 to -330 mV

Change of metabolic activity
- Growth rate
- Fermentation products (organic acids)
- H₂ production

The redox potential (ORP)

CO dehydrogenase (*R. rubrum*)

- Dimers
- Ni-Fe-S cluster
- Enzyme activity ORP-dependent

Initial CODH activity as a function of the redox potential (*in vitro* assay)

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Drennan et al. PNAS 98.21 (2001)

Heo et al. PNAS 98.14 (2001)

Ribbon drawing of the CODH dimers (Ni-Fe-S clusters indicated)

100% active!  inactive
The redox potential (ORP)

Example: 3-step bioprocess for the production of PHA with *R. rubrum*

1. Aerobic phase
2. Stop of the aeration => anaerobic phase
3. Start of aeration with syngas (anaerobic)
Flow cytometry for PHA quantification

=> Need for a reliable at-line quantification method that can be easily implemented in an automated sampling system

**Nile red**
- lipophilic fluorescent dye
- fast bleaching
- crystal formation during storage
- broad emission spectrum

**BODIPY 493/503**
- stain for neutral lipids, environment-independent
- no photo bleaching
- intense and sharp fluorescence

*R. rubrum* stained with BODIPY 593/503 (PHA, green) and SYTO 62 (DNA, red).
Scale bar = 10 µm. (S. Karmann)
Flow cytometry for PHA quantification

Dual staining:
BODIPY 493/503 → PHA and SYTO 62 → DNA

- Elimination of false positives
  (lipidic substrates, PHA released from lysed cells, cells without PHA, cell debris)
- No washing steps necessary!

Optimized parameters
1. Dilution buffer:
   dH$_2$O, PBS, 50% PBS, NaCl, Tris, MgCl$_2$
2. EDTA
3. Staining time
4. Staining temperature

S. Karmann
(manuscript accepted in J. Microbiol. Methods.)
Example: PHA production bioprocess with *P. putida* KT2440 on octanoic acid

![Graph showing OD600 and mean fluorescence over time](image)

Example: PHA production bioprocess with *P. putida* KT2440 on octanoic acid

![Graph showing SYTO 62 (DNA) and BODIPY (PHA) counts](image)
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![Graph showing time after inoculation vs OD and mean fluorescence.]

![Graph showing SYTO 62 (DNA) and BODIPY (PHA) fluorescence.]

*Example: PHA production bioprocess with *P. putida* KT2440 on octanoic acid.*

*Graph showing time after inoculation vs OD and mean fluorescence.*

*Graph showing SYTO 62 (DNA) and BODIPY (PHA) fluorescence.*

S. Karmann
Example: PHA production bioprocess with *P. putida* KT2440 on octanoic acid
Example: PHA production bioprocess with *P. putida* KT2440 on octanoic acid

[Graph showing OD$_{600}$ and mean fluorescence over time after inoculation]

Example:

PHA production bioprocess with *P. putida* KT2440 on octanoic acid

S. Karmann

SYTO 62 (DNA)  
BODIPY (PHA)
Example: PHA production bioprocess with *P. putida* KT2440 on octanoic acid
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![Graph showing OD600 and mean fluorescence over time.](image1)

- **SYTO 62 (DNA)**
  - Q1: 0
  - Q2: 88.3
  - Q3: 11.3
  - Q4: 0.36

- **BODIPY (PHA)**
  - Counts range from 10^2 to 10^7

S. Karmann
Example: PHA production bioprocess with *P. putida* KT2440 on octanoic acid
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![Graph showing OD600 and mean fluorescence over time after inoculation](image)

![Flow cytometry plot showing SYTO 62 (DNA) and BODIPY (PHA)](image)
Example: PHA production bioprocess with *P. putida* KT2440 on octanoic acid.

![Graph showing OD600 and mean fluorescence over time after inoculation.]

![Flow cytometry plot showing SYTO 62 (DNA) and BODIPY (PHA) counts.]

S. Karmann

311815 SYNPOL

Madrid, 2016-09-09

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Example: PHA production bioprocess with *P. putida* KT2440 on octanoic acid

![Graph showing OD600 and mean fluorescence over time after inoculation]

![Histograms of SYTO 62 (DNA) and BODIPY (PHA) fluorescence]
Example: PHA production bioprocess with *P. putida* KT2440 on octanoic acid
Example: PHA production bioprocess with *P. putida* KT2440 on octanoic acid

![Graph showing OD600 and mean fluorescence over time after inoculation](image)

- **SYTO 62 (DNA)**
- **BODIPY (PHA)**
Example: PHA production bioprocess with *P. putida* KT2440 on octanoic acid.
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**Graph:**
- **X-axis:** Time after inoculation [h]
- **Y-axis 1:** OD600
- **Y-axis 2:** Mean fluorescence

**Scatter plot:**
- Points represent OD600 and mean fluorescence over time.

**Fluorescence dot plot:**
- **X-axis:** SYTO 62 (DNA)
- **Y-axis:** BODIPY (PHA)

**Histogram:**
- **X-axis:** BODIPY (PHA)
- **Y-axis:** Counts
Flow cytometry for PHA quantification

Bioreactor

Aseptic sampling

Liquid handling system

Dilution to $10^6$ cells mL$^{-1}$

Dual staining BODIPY 493/503 & SYTO 62

Online flow cytometer

Determination of
• Total cell count
• PHA content

Information sent to IRIS (bioprocess control system)
Bioreactor process control

• The measurement tools must be adapted according to the bioprocess (e.g. aerobic/anaerobic, type of product,...)

• At the R&D level comprehensive measurements (as many as possible!) are essential to gain a deep understanding of the process.

• Real-time or near real-time data are necessary for a rapid feed-back control.

• Communication between the different measurement tools and the control system is needed!
Bioreactor process control for bacterial syngas fermentations:

2. The ‘Automation Point of View’

Aldo Vaccari
Team (automation part)

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Aldo Vaccari
Senior researcher
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Interested in our activities?
http://www.hevs.ch/fr/rd-instituts/institut-systemes-industriels/collaborateurs/vaccari-1680

Field of Expertise

- Measurement
- Automation (Mechatronics / Biosystems)
- Control (standard PID / advanced)
- Process simulation
- PLC, Matlab, LabVIEW Programming
- Robotics
Overview

- **Bioreactor control equipment** (general)
- **What is LabVIEW?**
- **Control loop** (general)
- **What is a PID controller?**
- **Electric signal categories**
- **What is LabVIEW Real Time and NI-CRIO platform?**
- **Bioreactors Layout** (Project Synpol WP3)
- **Overview of the Communication** (Project Synpol WP3)
- **Liquid Handling Software and hardware** (Project Synpol WP3)
Bioreactor control equipment (general)

- **Base Pump**
- **Acid Pump**
- **Air / Gas**
- **Feed Pump**
- **Sensors Transmitters** (signal Conditioning)
- **PLC & I/O cards**
- **HMI & Datalogging**
- **Ethernet / USB / RS232**
- **I/O signals Analog and digital**
- **Stirrer Motor**
- **Mass Spectrometer**
- **Level Sensor**
- **pH Sensor**
- **Dissolved O₂ Sensor**
- **Thermocouple**
- **Sparger**
- **Agitator**
- **Exit Gas Flow**
- **Inlet Air Flow**
- **Exit Liquid Flow**
- **Automatic Sampling System**
- **Online Analysis (flow cytometer)**

**SYNPOL’s 4th Annual Course**
“Biopolymers from bacterial fermentation of syngas”

SYNPOL’s 4th Annual Course
“Biopolymers from bacterial fermentation of syngas”
LabVIEW (short for Laboratory Virtual Instrument Engineering Workbench) is a system-design platform and development environment for a visual programming language from National Instruments.
Control loop (general)

- pH setpoint value
- pH measured value
- pH error value
- PLC
- Acid/Base pump command
- Bioreactor
- pH probe

Diagram:
- Reference
- Measured error
- Measured output
- System input
- System output
- Sensor
- Controller

Information:
- Setpoint
- Measure
- PID gains
  - proportional gain (Kp)
  - integral time (Ti, min)
  - derivative time (Td, min)
- Command

- dt (s)
- PD
- PI
- PID
What is a PID controller?

**A Proportional-Integral-Derivative controller** is a control loop feedback mechanism (controller) widely used in industrial control systems.

**Proportional or P-controller** gives output which is proportional to current error \( e(t) \). It compares desired or set point with actual value or feedback process value. The resulting error is multiplied with proportional constant to get the output. If the error value is zero, then this controller output is zero.

Due to limitation of p-controller where there always exists an offset between the process variable and set point, **I-controller** is needed, which provides necessary action to eliminate the steady state error. It integrates the error over a period of time until error value reaches to zero. It holds the value to final control device at which error becomes zero.

I-controller doesn’t have the capability to predict the future behavior of error. So it reacts normally once the set point is changed. **D-controller** overcomes this problem by anticipating future behavior of the error. Its output depends on rate of change of error with respect to time, multiplied by derivative constant. It gives the kick start for the output thereby increasing system response.
Electric signal categories

Analog Input/Output

An analog signal is a signal that varies continuously.
Analog input/output signals are most commonly voltage (0-10V) or current (4-20mA).

Digital I/O

Electrical signals that transfer digital data (on/off, high/low, 1/0) using a wire.
Used to transfer data:
To program devices or to communicate between devices (RS232 / USB / Ethernet).
What is a National Instruments Compact RIO?

The CompactRIO platform features a range of embedded controllers with two processing targets: (1) a real-time processor for communication and signal processing and (2) a user-programmable FPGA to implement high-speed control and custom timing and triggering directly in hardware. Eliminate the need for separate subsystems by connecting directly to sensors.
Development PC and CRIO communication

Once the application is developed, it can be compiled into the CRIO as an exe and it can operate independently without PC.
What is LabVIEW Real Time?

Real Time Response: The ability to reliably and, without fail, respond to an event or perform an operation, within a guaranteed time period.

Maximum Jitter

Desired Loop Time

Jitter Range

Maximum Jitter

Loop Iteration:
1
2
3
4
5

Loop Time (seconds)

ni.com/training
Generic OS vs Real Time OS

**General Purpose OS**
- Data acquisition
- Offline analysis
- Data presentation

**Real-Time OS**
- Closed loop control
- Time-critical decisions
- Extended run time
- Stand alone operation
- Increased reliability

Windows 10
OS X
CRIO
Bioreactors Layout (Project Synpol WP3)

Typhoon_1
Infors Labfors 13l
with CIP unit

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

Typhoon_2
Infors Labfors 3.6l

Infors Touchfors
Touch panel

Infors Iris 6.0
HMI

Hiden
Mass Spectrometer

Infors
Iris 6.0
HMI

Synpol
4th Annual Course
"Biopolymers from bacterial fermentation of syngas"
Overview of the Communication (Project Synpol WP3)

Bioreactor Control

Gas analyzer (MS)

INFORS HT

HIDCN ANALYTICAL

QGA Pro (LabVIEW USB driver)

PC1

IRIS

OPC DA to OPC XML DA Bridge

Flowcytometer (FC)

PC2

Telnet

Ethernet

NI shared variables

Fluid Handling

QGA Pro (LabVIEW USB driver)

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**BD Accuri Flow Cytometer (Project Synpol WP3)**

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):
Liquid Handling Software (Project Synpol WP3)
Liquid Handling Hardware (Project Synpol WP3)

- **Electric cabinet with CRIO**
- **Peristaltic pump**
- **Sample loop (250ul)**
- **FlowCytometer**
- **ON/OFF Valves**
- **Mixing Chamber on magnetic stirrer**
- **Complete Fluid Handling system**
- **HMI + Datalogging**

**FlowCytometer**

**HMI + Datalogging**

**ON/OFF Valves**

**Mixing Chamber on magnetic stirrer**

**Complete Fluid Handling system**

**Sample loop (250ul)**

**Peristaltic pump**

**Electric cabinet with CRIO**
Mixing Chamber Design (Project Synpol WP3)

Final Version:

- Silicon septum
  - problem with the air pressure inside the mixing chamber is solved.
  - support the syringes

- Syringes
  - the quantity of sample is more accurate.
Thank you for your attention