Real-time cell culture control in an integrated benchtop platform: implications for research and training

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Roadmap

Context: Needs for controlling the bioprocess in biomanufacturing, research, and training

Project goal: Development of a flexible integrated benchtop bioreactor platform
- Dynamic control of glucose and serine in prokaryotic culture
- Using an integrated bioprocess platform for teaching

Vision for the future: Interfacing analytics to the bioprocess provides an evolving toolkit for research, teaching and communication

Process Analytical Technology – “PAT”

FDA definition (Guideline for Industry, 2004)

“The Agency considers PAT to be a system for designing, analyzing, and controlling manufacturing through timely measurements (i.e., during processing) of critical quality and performance attributes of raw and in-process materials and processes, with the goal of ensuring final product quality…

... quality cannot be tested into products; it should be built-in or should be by design...”

It is important to note that the term analytical in PAT is viewed broadly to include chemical, physical, microbiological, mathematical, and risk analysis conducted in an integrated manner...

“PAT” tools

In the PAT framework, these tools encompass:
- Multivariate data acquisition and analysis
- Process analytical chemistry tools
- Endpoint monitoring and control tools
- Continuous process improvement and knowledge management tools
- An appropriate combination of these tools may apply to a single-unit operation, or to an entire manufacturing process, and its quality assurance
“PAT” goals for enhancing process efficiency and product quality

Reduction of production cycle times,
by integrating unit operations, together with in-situ, on-line or at-line measurements and controls

Improvement of operator safety and reduction of human error,
by increasing automation

Minimization or avoidance of rejects, with a move toward real-time product release

Increase of process efficiency and control of product attributes,
by facilitating continuous processing

Development of small-scale equipment & instruments for manufacturing, scaled-down models, and early screening

New “PAT” contributions (2005-2008): focus on biomolecules producers

• Process analytical technology in biochemical production (2005)
• Implementing an automated sampling system for mammalian cell culture systems (2007)
• Online monitoring of mammalian cell cultures (2008)

Bioprocess monitoring and computer control: key roots of the current PAT initiative

AMGEN (Biotech. Bioeng. 2008;100: 306-316)
Application of Process Analytical Technology toward bioprocessing: using on-line high-HPLC for making real-time pooling decisions for process chromatography

MEDIMMUNE (ACS Biotechnology Division 2008)
Assessment of platform vaccine process development and improvement of vaccine productivity through bioprocess optimization

BIOGENIDEC (ACS Biotechnology Division 2008)
Online process monitoring and feedback control for rapid development of better optimized cell culture processes

PFIZER (ACS Biotechnology Division 2008)
Performance evaluation of an automated bioreactor sampling system for mammalian cell cultivation

Early “PAT” contributions (2003-2005): focus on instrumentation and screening tools

Near-infrared spectroscopy as a process analytical tool - Part 1: Laboratory applications

6th ANNUAL CONGRESS OF CHEMOMETRICS (2003) & Chemometrics and Intelligent lab syst., 2004;
74(2): 269-275
Chemometrics in bioprocess engineering: process analytical technology (PAT) applications

Emergent FDA biodefense issues for microarray technology: process analytical technology

Automated bioprocess sampling and analysis

FLOWNAMICS (bioautomation, 2005; 2: 49-53)
Sampling probe

Near-infrared spectroscopy for Process Analytical Chemistry: theory, technology and implementation

On-line liquid chromatography as a PAT for monitoring and control of biotech processes

Recent “PAT” contributions (2009-2014): old & new players

ACADEMIC CONTRIBUTIONS
Advances in on-line monitoring and control of mammalian cell cultures: Supporting the PAT initiative

• Automated measurement and monitoring of bioprocesses: key elements of the (M)‐C‐S strategy
• Applying mechanistic models in bioprocess development

Bioreactor monitoring with spectroscopy and chemometrics: a review

INDUSTRY CONTRIBUTIONS
BEND RESEARCH
Poster on on-line modular automated sampling technology, presented at the C&G Conference (2014)

BOERINGHER
Advancing bioprocess development by system-level data analysis and integration of omics data: (In: Genomics and systems biology of mammalian cell culture, 2013; Vol. 127)

GENZYME
Presentation on integrated continuous bioprocessing (CCB, 2014)

DISPOSABLE (SINGLE-USE) TECHNOLOGY DEVELOPERS
UPS & DSP, such as PBS Biotech, Eppendorf, Hyclone, Sartorius, EMD Millipore, ATMI-Pall, GE Sensors, such as Finesse, Aber, Hamilton, PediMax, Polkadot Tech.
Improving through automation

- Process design, safety & repeatability
- Product quantity, quality
- Production cost

How to approach automation?

- Gain process knowledge (e.g., cell, medium, environment)
- Assess key parameter which can be or should be controlled and automated

Bioprocess design and control options

- Manual or automated sampling, treatment and analysis
- Feedback or Predictive control
- Off-line analytics (e.g., for glucose, amino-acids)
- At-line analytics (e.g., for dissolved O₂, Capacitance)

Manual sampling and analysis: glucose consumption rate as a temperature control trigger

Real-time glucose consumption rate (GCR) as a trigger to temperature switch during 60-day perfusion CHO culture (Meuwly et al., 2006)

\[ \text{GCR} = f(V, M_{\text{packed bed}}, \text{glucose}) \]

Successful bioprocess control on multiple sites based on manual sampling and off-line analysis
GCR triggers T shift down
(Meuwly et al., 2006)

Discussing merits of automation
(Meuwly et al., 2006)

"... the benefits of on-line regulation have to be analyzed carefully...

... the GCR approach is not prone to automation breakdown or programming errors..."

Assessing feedback control and feeding strategies
(Lu et al., 2012)

Real-time analysis of metabolites and product in CHO fed-batch culture, through 3 feeding strategies targeting the maintenance of 4 to 6 g/L glucose in the bioreactor:

1. Autosampler and at-line glucose and viable cell concentrations dynamic feeding

2. No autosampler and off-line glucose concentration: manual-adjusted feed, every 6 h,

3. No autosampler and no real-time analysis: bolus feeding: every 3 days at 6.7% initial culture volume
Effect of feeding strategy on cell growth  
(Lu et al., 2012)

![Graph showing the effect of feeding strategy on cell growth.](image)

Feeding strategy and glucose concentration  
(Lu et al., 2012)

![Graph showing the feeding strategy and glucose concentration.](image)

Feeding strategy and product titer  
(Lu et al., 2012)

![Graph showing the feeding strategy and product titer.](image)

Bioprocess design and control options

![Diagram illustrating bioprocess design and control options.](image)
**Capacitance-based probe**

- Bioreactor envelope
- Tip of probe
- Non-polarized dead cells
- Polarized viable cells
- Electric field

**Predictive-control feeding on product titer**

Lu et al. (2012)

- Product titer (g/L)
- Run time (d)
- Dynamic feed (capacitance)
- Bolus, every 3 days

**Predictive-control vs. bolus feeding on product quality**

Lu et al. (2012)

- Error bars = analytical accuracy of measurements
- Dynamic feed (capacitance)
- Bolus, every 3 days

**Predictive-control & feedback-control feedings on product titer**

Lu et al. (2012)

- Product titer (g/L)
- Run time (d)
- Dynamic feed (glucose)
- Bolus, every 3 days
Integrated benchtop bioprocess platform and control options

Design control strategy (simple feedback control)

Process action

Compare PV, AV, and SP

Use process knowledge

Autosampler

Analytics

PVAV and SP

Desired set point (SP) of selected automation variable (AV)

Integrated benchtop platform for research and teaching: schematic diagram

The sanitary interface: a key element for maintaining asepsy

Step 1. Prime and sample

Waste

Reactor Sample to ARS-M

Step 2. Instrument analysis

Waste

Sample

Closed Valve

Cleaning Reagents

Step 3. Cleaning Cycle

Waste

Reactor Sample

Sample ARS-M

Step 4. Purge

Waste

The sanitary interface: a key element for maintaining asepsy

Execute Control Strategy

Add glucose, e.g. 5 mL of 100 g/L stock

Calculate

Set Point

DASGIP Control

Feed Pump

Reactor Sample

Communicate

Analyze

OPC Client/Server

Glucose analyzer

ARS-M autosampler
Integrating benchtop platform for research and teaching: overview (Hamel et al., 2009)

Manual or automated sampling, treatment and analysis

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Bioreactor feeding study

**Biological platform**

**Fermentation**

Batch culture of BL21 E.coli in liter-sized traditional stirred bioreactor - casamino-acid medium with glucose

Product is Green Fluorescent Protein (GFP)

**HPLC**

Ortho-phthalaldehyde/9-fluorenylmethylchloroformate (OPA/FMOC) derivatized amino acid analysis (C18 column, Room T, 5 µL injection)

About 30 min per analysis, and 1.5 hour total between samples

**Study objectives**

- Glucose feeding control (goal: 2 g/L)
- Amino-acid (AA) analysis, and control (goal: limiting AA > 250 mg/L)

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Kinetics of E.coli growth and glucose in batch culture - traditional bioreactor

From stepwise to “steady-state” control
Amino-acid (off-line) analysis during *E. coli* batch culture

No feedback control

Serine depletion in BL21 *E. coli* culture

Serine

0 hr

4 hr

24 hr

OPC vs. off-line amino-acid analysis during *E. coli* culture

With feedback control

Green fluorescent protein productivity gain with OPC-control of serine in *E. coli* culture

With serine feeding

33%

No serine feeding

With GFP productivity (μg/ml), Specific Productivity (μg/ml/OD)

FB GFP (μg/ml)

FB GFP/OD

Con GFP (μg/ml)

Con GFP/OD

FB OD600

Con OD600

Process Time (Hours)

OD 600
Integrative benchtop platform for research and teaching MIT (2009)

- **Feedback control**: Manual or automated sampling, treatment and analysis
  - Manual sampler
  - Off-line analytics (e.g., for glucose, amino-acids)
  - With cell retention device
  - Autosampler
  - At-line analytics (e.g., for glucose, amino-acids)

**Working principle of air-lift disposable bioreactor**

Glucose feedback control with Labview-based approach

1. Cell sample
2. Cell-free sample
3. Peristaltic pump is turned ON: Glucose is added
4. Glucose datum
5. Signal
6. Target set point

E. coli culture in air-lift (single-use) bioreactor: glucose control (3 g/L setpoint)
Using an integrated bioprocess platform for teaching

The goal: teaching locally and world wide

The audience: students, teachers and professionals

The teaching frameworks:
1. Experimental lab
2. Simulation lab
3. Remote lab

Assessing the 3 lab formats (relative comparison)

<table>
<thead>
<tr>
<th>Attribute or experience</th>
<th>Experimental lab</th>
<th>Simulation lab</th>
<th>Remote lab</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interaction level between student and process, and between team members</td>
<td>High</td>
<td>Low</td>
<td>Medium</td>
</tr>
<tr>
<td>Capital cost</td>
<td>High</td>
<td>Low</td>
<td>Medium</td>
</tr>
<tr>
<td>Operating cost</td>
<td>High</td>
<td>Low</td>
<td>Medium</td>
</tr>
<tr>
<td>Convenience and ease to students</td>
<td>Medium</td>
<td>Medium</td>
<td>High</td>
</tr>
<tr>
<td>Student preference</td>
<td>High</td>
<td>Low</td>
<td>Medium</td>
</tr>
<tr>
<td>Learning outcomes</td>
<td>?</td>
<td>?</td>
<td>?</td>
</tr>
</tbody>
</table>

Sonnenwald et al., 2003
Lindsay & Good, 2005
Corter et al., 2007, 2011

Learning outcomes [total test score] between hands-on, simulation and remote labs (based on Corter et al., 2011)

<table>
<thead>
<tr>
<th>Data collection mode</th>
<th>Individual</th>
<th>Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Typical mechanical engineering course</td>
<td>hands-on</td>
<td>sim</td>
</tr>
<tr>
<td>Score</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Error bar=1SD</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Using the integrated platform for hands-on and remote teaching

Sharing process knowledge, control and data over multiple sites

Bioreactor technology
- In-situ and soft sensors
- Chemical and bioeng. concepts
- Process monitoring and control

At-line interfaces
- Asepsy
- Communication protocols
- PAT and regulatory needs

Analytics
- Cell metabolism
- Feeding strategies for advanced control

New and potential analytical tools for integrated bioprocess research platform

**Based on the last decade**
- Bioreactors: traditional and disposable; macro-to micro-scale
- In-situ instruments: Raman, FTIR, NIRS, capacitance, fluorescence, bio-, electrochemical and optical probes, sensors interfaced with microfluidics
- On-line soft sensors
- At-line instruments: Autosampler, flow cytometry, SPR, HPLC, CE, glucose, process module

**New or on the horizon**
- In-situ: glucose
- Off-line instruments: NMR, lensless imaging

**Recent: In-situ glucose monitoring**
- Stainless steel probe that measures the refractive index directly in the bioreactor (Sparrow et al., 2009)
- Gamma-radiated enzymatic-based glucose sensor (CE Webiste, 2014)

**On the horizon: lensless imaging**
- Kenavan et al. (2013)
- Source: CEA-LEITI, 2014
On the horizon: integrated analytical technologies

MicroRAMAN combined with lensless imaging and scattering microscopy (Strola et al., 2013)

The combination provides the precise bacterium localization, its chemical composition and a morphology description

Capacitance and lensless imaging

Soft sensors

The evolving integrated bioprocess platform

Sensors and analytics

Traditional & macro

In-situ & at-line

Soft sensors

Novel & micro

In-situ & ex-situ

Basic & advanced controls

Sensors and analytics

Manual or automated sampling, treatment and analysis

Feedback Predictive control

Off-line analytics (e.g., for glucose, amino-acids)

Manual sampler

At-line analytics (e.g., for glucose, amino-acids)

With or without cell retention device

No sample or sample treatment

Dissolved O₂, Capacitance

Gas O₂

Cell culture vessel

 Autosampler

Designing for diverse users

Designers and vendors of bioprocessing, analytical sensors and automation systems need to consider the diverse international settings and regulatory requirements of the following groups:

- Educators
- Researchers
- Students and trainees
- Professionals
- Regulators

A vision for the future
Spirulina for food production

- Addressing Malnutrition

- Current Reactors
  - Open system:
    - Lakes, tanks, ponds

- Problems
  - Low growth rate:
    - 4-10 g/m²·day
  - Requires large area
  - Contamination

Women Harvesting Spirulina off Lake Chad
Obtained from www.new-agri.co.uk, 2008

High-tech culturing system

- Illuminated stirred reactors
- CO₂ Control system

Low-tech (cheap) air-lift reactor/biodigester system

Conclusion

Under the PAT framework, the need for controlling and improving the bioprocess has been addressed by industry and academia

Flexible integrated benchtop bioreactor platform useful for:
- Research and process development (e.g., automated analysis over entire process, feedback control multiple parameters)
- Manufacturing supervision (e.g., real-time monitoring of process progress, and of product attributes, data for compliance purpose)
- Teaching concepts relevant to diverse science and engineering disciplines, and to regulation
- Enhancing communication from both the local and global viewpoints

Acknowledgments

- Groton Biosystems
- DasGip/Eppendorf
- Cellexus
- National Instruments
- YSI
- Agilent Technologies
- MIT