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 for 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

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

FOSS NIR SYSTEMS (Spectroscopy, 2003; 18(11): 32-35)
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

GE (Expert Review of Mol. Diag, 2004; 4(6): 779-781)
Emergent FDA biodefense issues for microarray technology: process analytical technology

GROTON BIOSYSTEMS (Gen. Eng. News, 2004; 24(14): 54-56)
Automated bioprocess sampling and analysis

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

ABB & Adv Solut (in Process Analytical Technology (ed K. A. Bakeev), Blackwell Pub., 2005
Near-infrared spectroscopy for Process Analytical Chemistry: theory, technology and implementation

DIONEX CHEM. AND ELI LILLY (J. Biotech.; 2005; 118(1): S35-S36)
On-line liquid chromatography as a PAT for monitoring and control of biotech processes

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

GENENTECH (ACS National Conference, 2005, 2007, and ACS Biotechnology Division 2008)
- 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)

MERCK (*Biotech. Bioeng*. 2006;95(2):226-61)
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

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

ACADEMIC CONTRIBUTIONS

Biotech. Adv. , 2009; 27(6): 726-732
Advances in on-line monitoring and control of mammalian cell cultures: Supporting the PAT initiative

Measurement, Monitoring, Modelling and Control Book Series: Adv. in Biochem. Eng.-Biotech ; 2013: 132
- Automated measurement and monitoring of bioprocesses: key elements of the (MC)-C-3 strategy
- Applying mechanistic models in bioprocess development

Anal. Bioanal. Chem., 2013: 404(4): 1211-1237
Bioreactor monitoring with spectroscopy and chemometrics: a review

INDUSTRY CONTRIBUTIONS

BEND RESEARCH
Poster on at-line modular automated sampling technology, presented at the CCXIV Conference (2014)

BOERINGHER
Advancing biopharmaceutical process 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 (CCXIV, 2014)

DISPOSABLE (SINGLE-USE) TECHNOLOGY DEVELOPPERS
UPS & DSP, such as PBS Biotech, Eppendorf, Hyclone, Sartorius, EMD Millipore, ATMI-Pall, GE
Sensors, such as Finesse, Aber, Hamilton, PreSens, Polestar Tech.

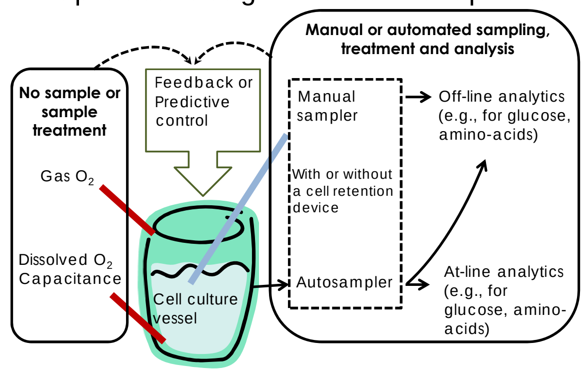
Improving through automation

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

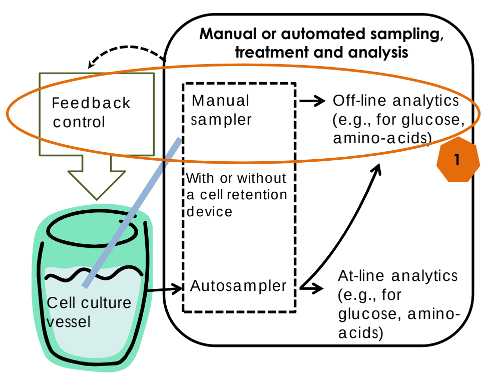
How to approach automation?

- 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



Bioprocess design and control options



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

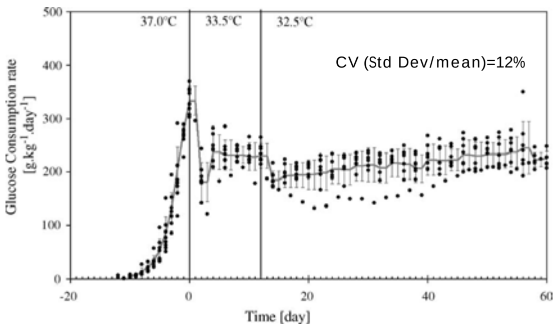
Real-time glucose consumption rate (GCR) as a trigger to temperature switch during 60-day perfusion CHO culture (Meuwly et al., 2006)

$GCR=f(V, M_{packed\ bed}, glucose)$

Successful bioprocess control on multiple sites based on manual sampling and off-line analysis

GCR triggers T shift down

(Meuwly et al., 2006)



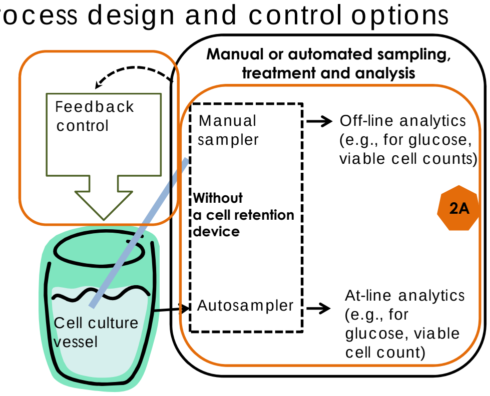
Discussing merits of automation

(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…"

Bioprocess design and control options



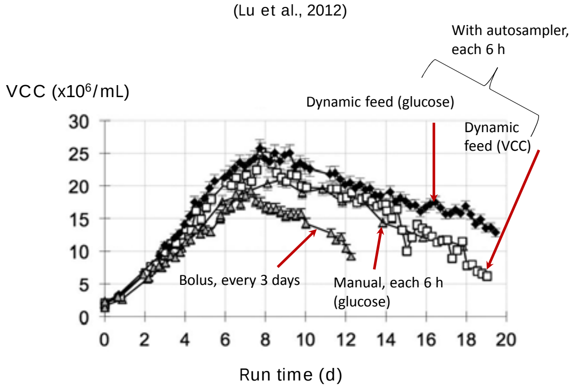
Assessing feedback control and feeding strategies

(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:

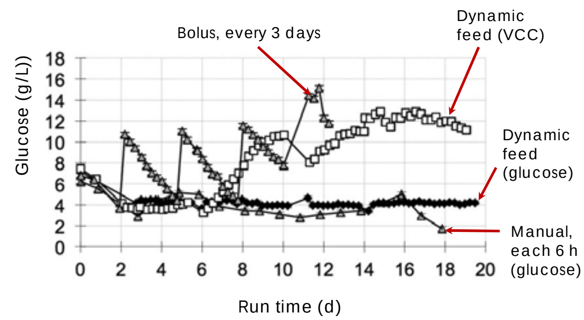
1. Autosampler and at-line glucose and viable cell concentrations: dynamic feeding
2. No autosampler and off-line glucose concentration: manual-adjusted feed, every 6 h,
3. No autosampler and no real-time analysis: bolus feeding: every 3 days at 6.7% initial culture volume

Effect of feeding strategy on cell growth
(Lu et al., 2012)



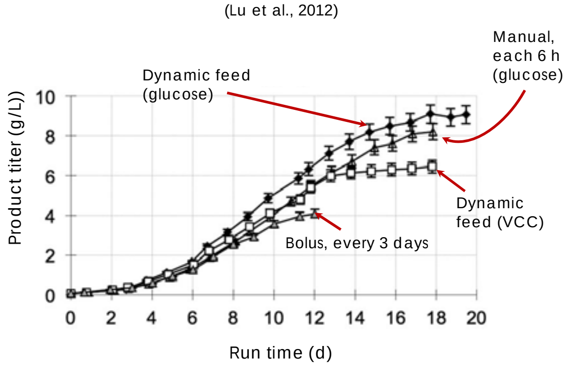
Biopharmaceutical Process and Quality Consortium – UMass Lowell – J.-F. Hamel - May 29, 2014

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



Biopharmaceutical Process and Quality Consortium – UMass Lowell – J.-F. Hamel - May 29, 2014

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



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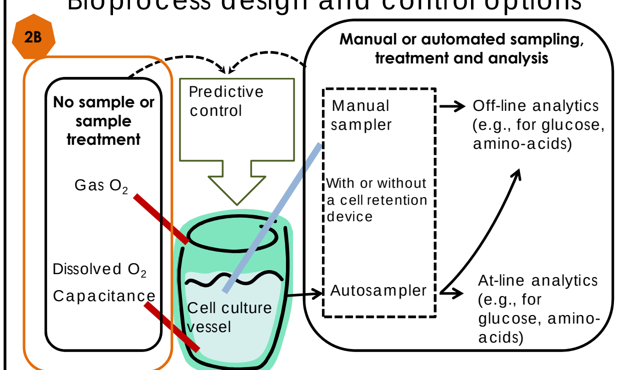
Bioprocess design and control options



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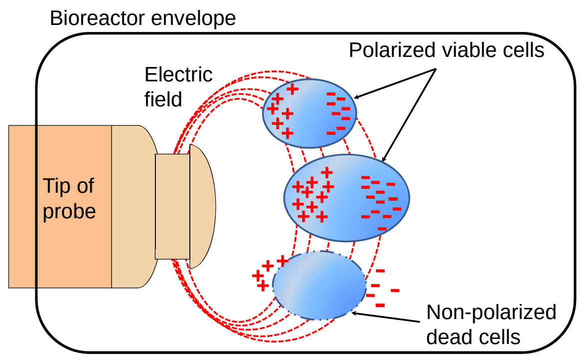
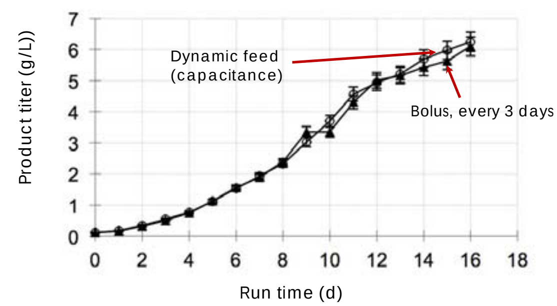
Capacitance-based probe



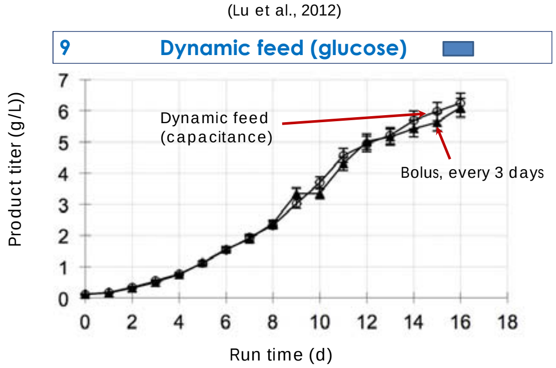
Predictive-control feeding on product titer

(Lu et al., 2012)



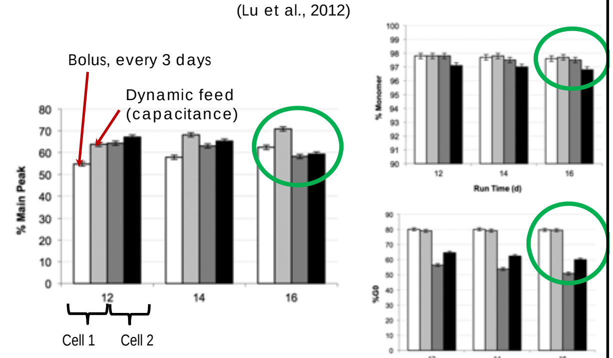
Predictive-control & feedback-control feedings on product titer

(Lu et al., 2012)



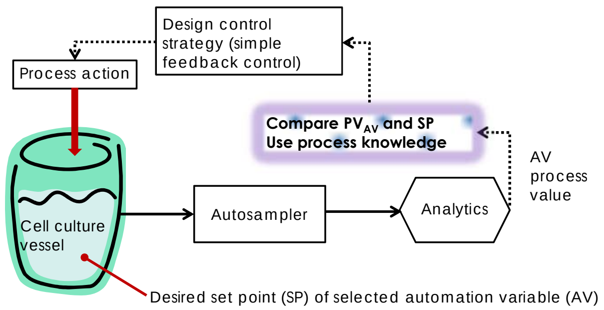
Predictive-control vs. bolus feeding on product quality

(Lu et al., 2012)

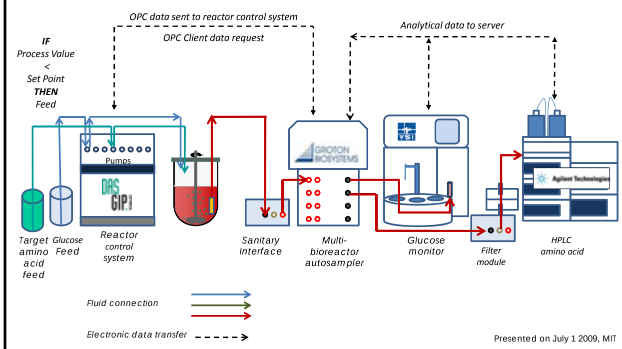


Error bars = analytical accuracy of measurements

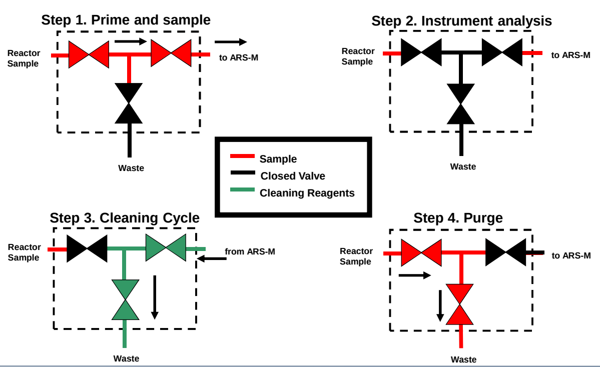
Integrated benchtop bioprocess platform and control options



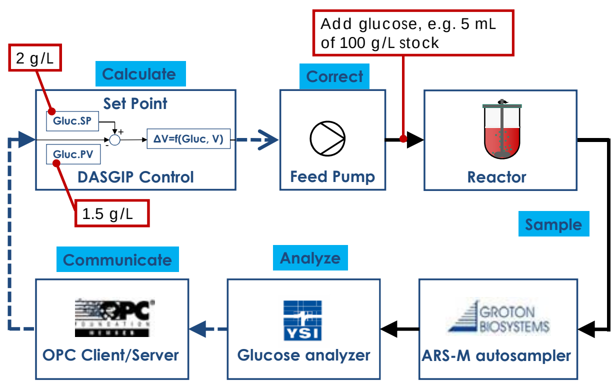
Integrated benchtop platform for research and teaching: schematic diagram



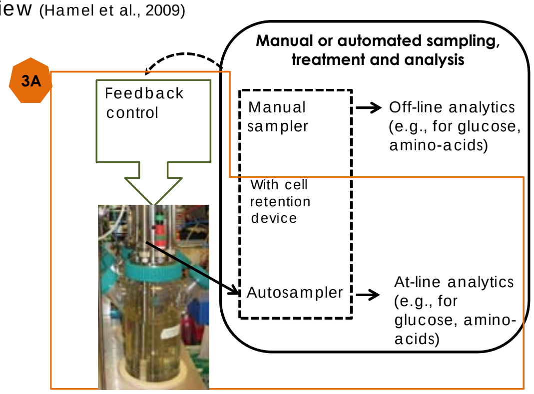
The sanitary interface: a key element for maintaining asepsy



Execute Control Strategy



Integrated benchtop platform for research and teaching: overview (Hamel et al., 2009)



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-phthal-aldehyde/9-fluorenyl-methylchloroformate (OPA/FMOC) derivatized amino acid analysis (C18 column, Room T, 5 µL injection)

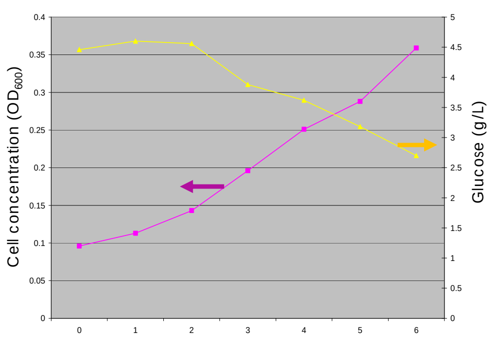
About 30 min per analysis, and 1.5 hour total between samples

Study objectives

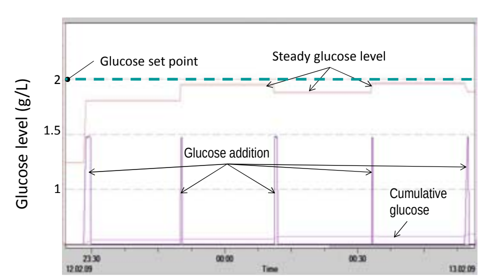
Glucose feeding control (goal: 2 g/L)

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

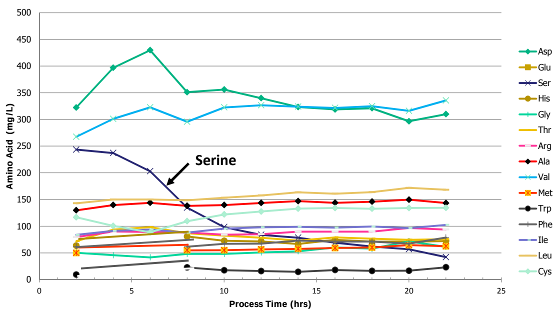
Kinetics of *E.coli* growth and glucose in batch culture – traditional bioreactor



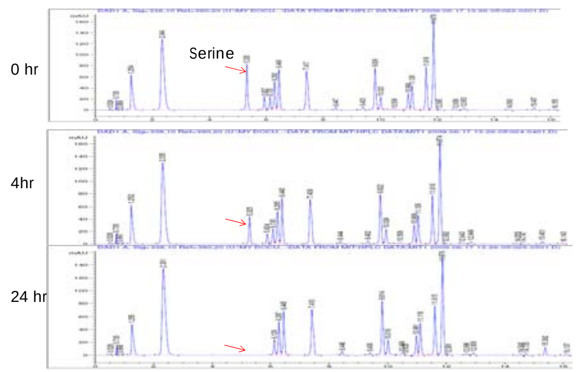
From stepwise to "steady-state" control



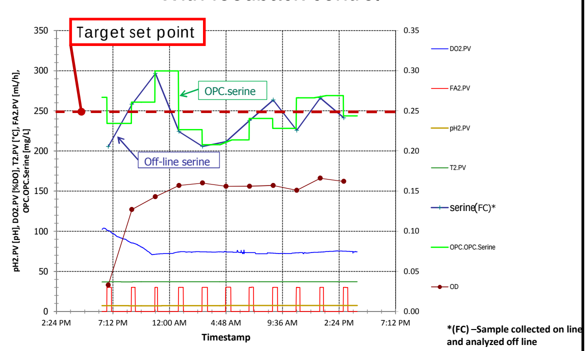
Amino-acid (off-line) analysis during *E. coli* batch culture No feedback control



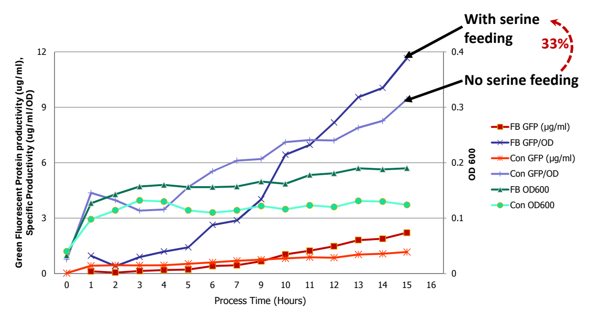
Serine depletion in BL21 *E. coli* culture



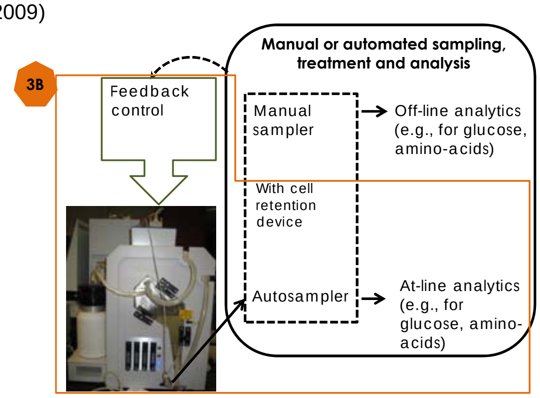
OPC vs. off-line amino-acid analysis during *E. coli* culture With feedback control



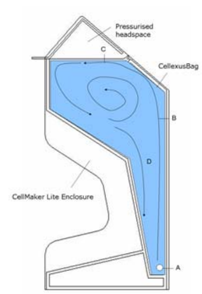
Green fluorescent protein productivity gain with OPC-control of serine in *E. coli* culture

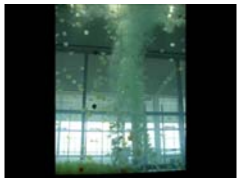


Integrated benchtop platform for research and teaching
MIT (2009)

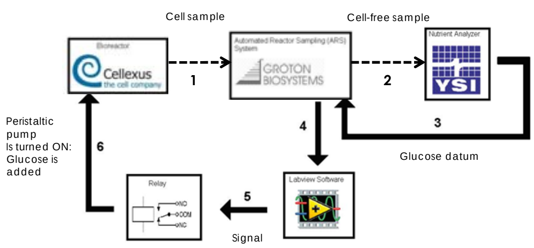


Working principle of air-lift disposable bioreactor

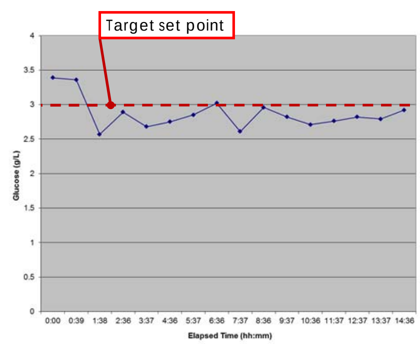




Glucose feedback control with Labview-based approach



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

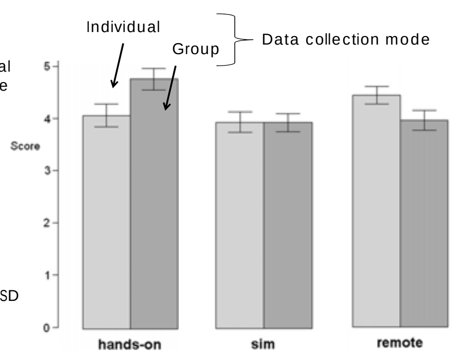
Assessing the 3 lab formats (relative comparison)

Attribute or experience	Experimental lab	Simulation lab	Remote lab
Interaction level between student and process, and between team members	High	Low	Medium
Capital cost	High	Low	Medium
Operating cost	High	Low	Medium
Convenience and ease to students	Medium	Medium	High
Student preference	High	Medium	Medium
Learning outcomes	?	?	?

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)

Typical mechanical eng. course



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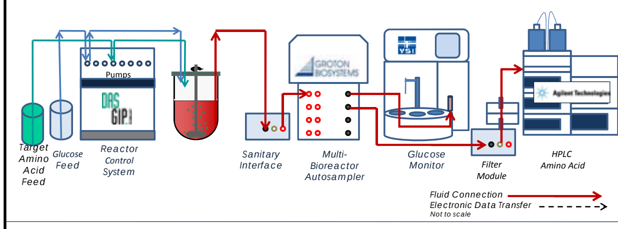
Using the integrated platform for hands-on and remote teaching

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, NIR, **capacitance**, fluorescence, bio-, electrochemical and **optical probes**, **sensors interfaced with microfluidics**

On-line soft sensors

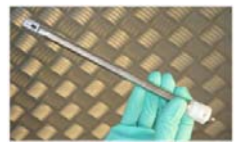
At-line instruments: **Autosampler**, flow cytometry, SPR, HPLC, **CE**, glucose, process module

New or on the horizon

In-situ: **glucose**

Off-line instruments: NMR, **lensless imaging**

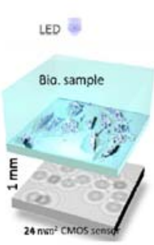
Recent: *In-situ* glucose monitoring

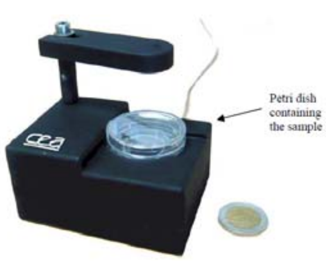


Stainless steel probe that measures the refractive index directly in the bioreactor (Sparrow et al., 2009)

Gamma-radiated enzymatic-based glucose sensor (Cit Website, 2014)

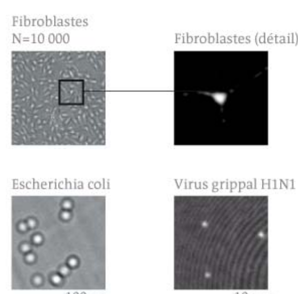
On the horizon: lensless imaging





Kesavan et al. (2013)

On the horizon: lensless imaging (continued)



Source: CEA-LEITI, 2014

On the horizon: integrated analytical technologies

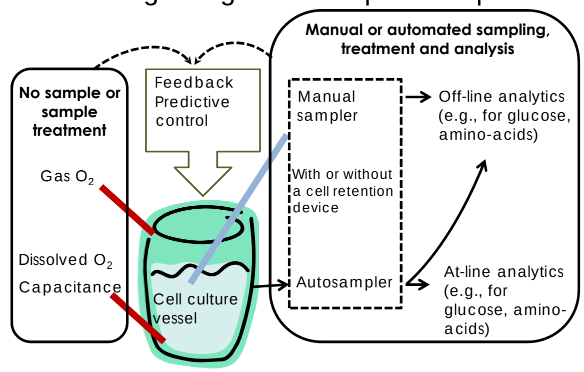
MicroRAMAN combined with lensless imaging and scattering microscopy (Strola *et al*., 2013)

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

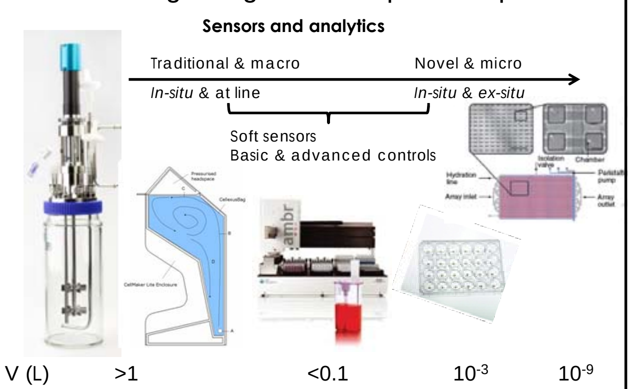
Capacitance and lensless imaging

Soft sensors

The evolving integrated bioprocess platform



The evolving integrated bioprocess platform



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/m^2- day
  - Requires large area
  - Contamination

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

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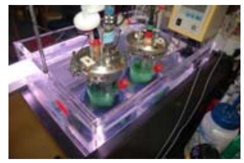
High-tech culturing system

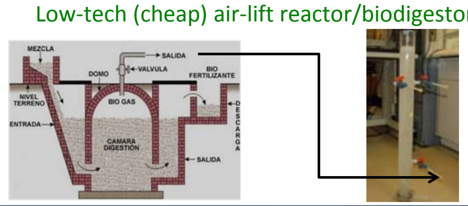


Iluminated stirred reactors

CO_2

Control system

Low-tech (cheap) air-lift reactor/biodigestor 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

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Agilent Technologies
MIT