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Editorial

Measurement Technologies for Upstream and Downstream Bioprocessing

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This special issue is devoted to new developments in measurement technologies for upstream and downstream bioprocessing. The recent advances in biotechnology and bioprocessing have generated a number of new biological products that require more qualified analytical technologies for diverse process analytical needs. This includes especially fast and sensitive measurement technology that early in the process train can inform on critical process parameters related to process economy and product quality and that can facilitate ambitions of designing efficient integrated end-to-end bioprocesses [1–3]. The flow of information about critical parameters should allow enhancement of productivity and better utilization of materials between process stages and unit operations. In integrated processes such information flows need to be faster than in conventional processes in order to allow the intended continuity (Figure 1). This is possible only with analytical monitoring methods based either on real-time or in-line sensor technology, on simple and compact bioanalytical devices, or use of advanced data prediction methods [4–7].

Although the Process Analytical Technology initiative, originally outlined by the pharmaceutical regulatory agencies, aims for pharmaceutical products, its relevance to bioprocess engineering is wider [8]. It may comprise all kinds of bioprocesses, from productions of small molecules, proteins, or cells for food and drugs, as well as commodity products. The aspects of quality-by-design, defining the design and control space for critical quality and process parameters are relevant beyond pharmaceutical processes. Industry, however, must achieve this quality within such economical frames that the production cost can cover. If quality cannot be achieved at a cost which is within the actual market value including coverage of upfront costs, sustainability of the product is lost. This calls for measurement technology which ensures both quality criteria and manufacturing efficiency.

The eight research articles in this special issue present novel approaches for advancing monitoring and control technology in these directions.

One angle of approach is about advancing the measurement principle itself to enhance sensitivity and selectivity when analysing critical parameters and attributes of cells and biomolecules, including viruses, proteins, and metabolites during bioprocessing.

This is successfully done with online digital holographic microscopy when monitoring a bioreactor culture with baculovirus-infected insect cells (Sf9 cells) that produce a recombinant adeno-associated virus. The digital holographic microscopy has the capacity to resolve in real time from samples withdrawn culture such important features as the viability of the insect cells and the titre of the produced virus [9]. With this information generated prior to subsequent downstream processing, efficient process integration can be expected.

Another novel measurement principle presented is nano-plasmonic sensing. This novel fibre-optical sensor technology allows rapid measurements of antibody (IgG) titres in bioprocesses [10]. The sensor is based on a combination of the optical effect of localized surface plasmon resonance with robust single-use Protein A-modified sensor chips to detect IgG molecules. The chip is housed in a flexible flow cell close to the process. This in-line technology has the capacity to be tailored to detect a variety of product molecules and their

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variants, either at early upstream stage or in later downstream stages for adaptation of the stages of benefit for integrated bioprocesses.

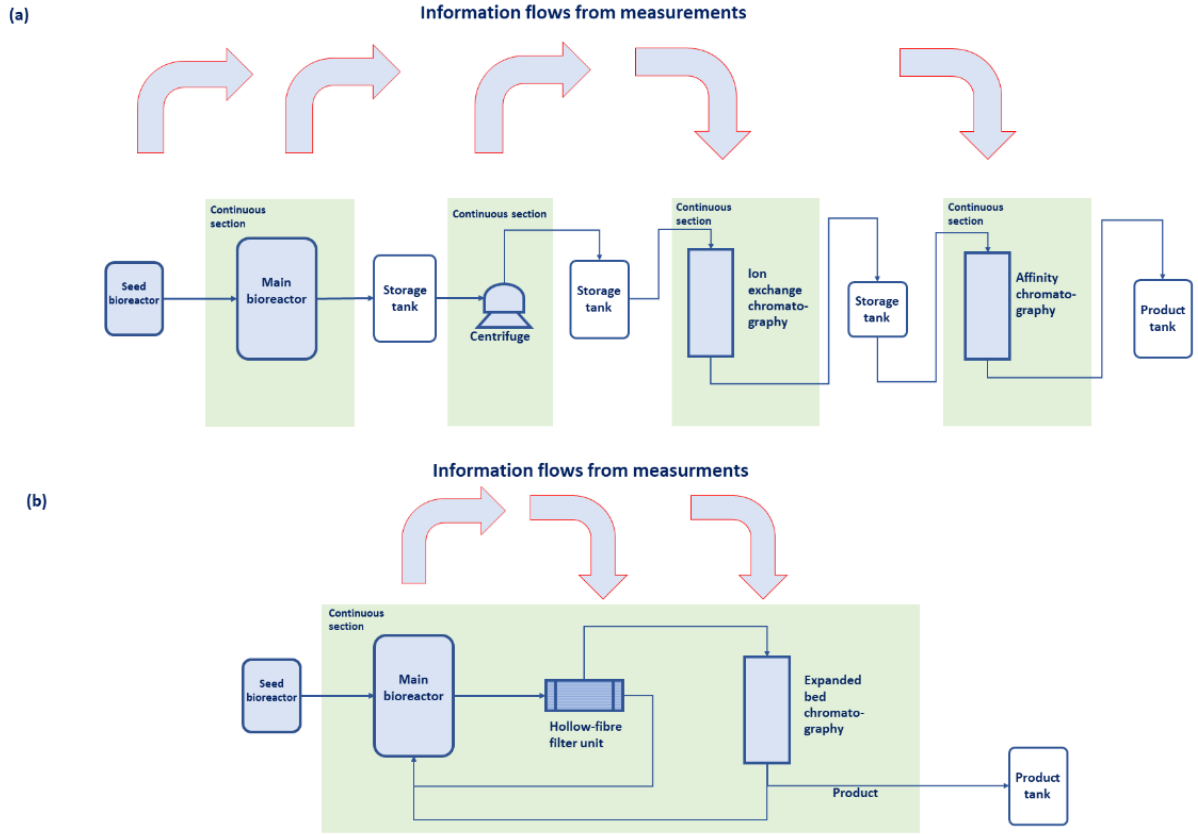

Figure 1. Bioprocess integration requires measurement technology that allows continuity and just-in-time information flow. (**a**) A conventional bioprocess with separate unit operations, (**b**) an end-to-end bioprocess with integrated and recycled flows between units. Depicted unit operations are examples.

Spectroscopic measurement technology can be developed further for at-line applications with the help of better spectral analysis methods to predict requirements in forthcoming stages. An example of this is high-throughput Raman spectroscopy microscopy using a spectral data analysis workflow to replace off-line analytics [11]. Promising results are shown for upstream applications with two mammalian cell lines that express different therapeutic proteins and demonstrate at-line monitoring of a high-throughput micro-bioreactor setup. This paves the way for improving process development and operation.

Another example of using established sensor technology for solving urgent bioanalytical needs is presented with in-line dielectric spectroscopy. Again, an insect cell-baculovirus expression vector system for large scale recombinant adeno-associated virus production is used, where the dielectric spectroscopy continuously monitors the production of the recombinant virus in the bioreactor [12]. As critically important when producing virus in insect cells, the cell concentration is monitored, and the infection time and cell viability at harvest are estimated with the purpose to enhance virus productivity and product quality. The use of in-line dielectric spectroscopy opens up for improving the robustness and control of the virus production.

A third example of exploiting an established methodology with smart computations is shown with size exclusion chromatography. Critical information on the release of impurity in *Penicillium chrysogenum* culture is captured from advanced data analysis [13]. The information is found in the ultraviolet chromatograms through fingerprinting principal component analysis to descriptively analyse the process trends. Prediction models using

partial least squares, orthogonal PLS, and principal component regression made it possible to predict the culture viability with model accuracies of 90% or higher.

Critical information can also be generated from pH measurements performed offline. A good example is presented where pH probe signals from bioreactors are corrected after the sterilization operation, but also to compensate for signal drift [14]. This novel non-invasive method to determine pH and pCO2 in bioreactors can be carried out without offline measurements by computation of the chemical correlation between carbon dioxide in the gas phase, dissolved carbon dioxide, bicarbonate, and dependent proton concentrations. The method enables accurate determination of the true pH in the bioreactor without sampling.

Convenient offline sampling of critical process data can also be achieved by employing new sensor fabrication technology. An example of this is shown with screen-printed enzyme-based electrochemical sensors for lactate monitoring in bioreactors [15]. These sensors have huge potential to enable low-cost off-line monitoring of, for example, overflow metabolites in bioprocesses. Here, the design of such a single-use electrochemical biosensor is evaluated. Several aspects of its fabrication and use are addressed, such as the importance of enzyme immobilization, stability, shelf-life, and reproducibility of the sensor.

Bioprocess computation of measurement signals requires successful integration and automation of the analytical procedures. Although well established in industry, pivotal improvements are required to address process analytical goals [16]. The shortcomings of automation are much due to the difficulty with the performance of the sampling procedure, sample preparation, and sample transfer to analysers; and very importantly, to correlate all data with the process and the sampling times. This is challenged in a study with an automated sampling system where the performance of data management software was performed with HPLC for measurement of vitamins and amino acids in combination with a biochemical analyser.

In essence, new process analytical technologies are permanently seeing the daylight. The methods highlighted in this special issue add new resources to bioprocess technology in line with the current industrial needs and where analytical principles are refined with new computational capacities and technological advancements.

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