Cytoprom
cell process monitoring

Application Note

Optical cell process monitoring without air bubble interference

A novel cell process monitoring system allows on-line measurements of growth curves to high cell densities, in real time, without bubble interference. It is now possible to obtain accurate spectral data and automate procedures in fermentation.

The importance of fermentation

Fermentation is a global industry with a turnover of hundreds of billions of Euros annually. Antibiotics, enzymes, amino acids, vitamins, organic acids, biodegradable plastics, polysaccharides, pharmaceuticals, textiles, laundry detergents, personal care products, and processed foods & drinks are some of the products produced by fermentation at a large scale. Optimization of fermentation protocols is critically dependent on the ability to measure and control key parameters. On-line analysis and control is essential for the development of efficient and reproducible bioprocesses and eliminates the potential loss of sterility. Another advantage is that closed control loops and automated procedures can be developed because on-line measurements allow an immediate response.

The challenge is to provide on-line sensors with a good lifespan that can withstand harsh bioprocess conditions, remain stable for the process duration, and offer a suitable working range. The incentives for improved analysis and control are increased efficiency, productivity and reproducibility - it saves money! Whereas temperature, pH and dissolved oxygen use well developed technologies, the monitoring of cell densities and product formation remain underdeveloped.

On-line biomass measurements

Direct measurement of turbidity remains the favourite method for estimating biomass because the technique is fast and easy to perform. This approach can now be used in on-line applications due to advances in fiber-optics, fiber optic probes that withstand in-place sterilisation and spectrometers. However, a major problem in optical measurements is the interference of air bubbles in aerated and agitated fermenters (**Fig. 1**). Air bubbles are significantly smaller in diameter than the optical path length of probes. When an air bubble passes through the optical window it causes scattering of light through reflection and refraction. At low cell densities scattering will be the dominant effect and will lead to a systematic overestimation of the density as less light passes through the optical window. In addition, large fluctuations of the signal will occur as a consequence of air bubbles passing through the optical window stochastically, leading to a large amount of random variation in the measurements. At high cell densities, where absorbance by the cells is at its greatest, the air

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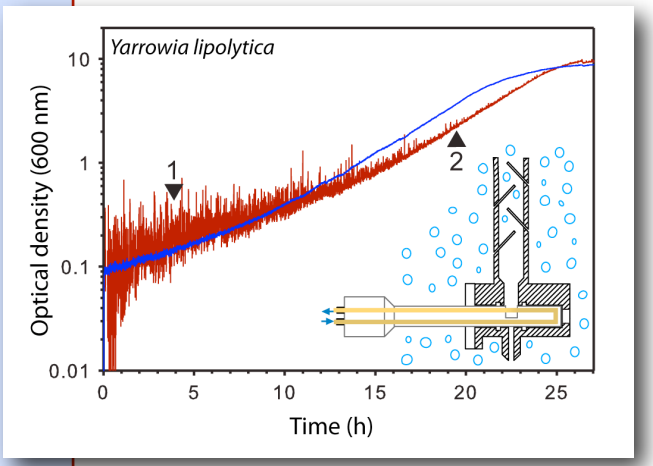

Fig. 1: Growth curves measured simultaneously in the presence (red) or absence of air bubbles (blue), which were removed by the Cytoprom bubble excluder. At low cell densities (point 1) scattering leads to an overestimation of cell density and large fluctuations. At high cell densities (point 2) absorbance by cells dominates, but the cell culture is displaced by bubbles and the cell density is underestimated.

bubbles displace part of the cell culture, meaning that the absorbance by cells for a given light path is less than when air bubbles are absent. This effect leads to a systematic underestimation of the cell density.

Most sensors currently available are strongly affected by air bubble interference, but even when their principle technologies are not affected by bubbles directly, the mere presence of bubbles may change the properties of the specimen by displacement in an unpredictable way, as explained above. If you cannot measure without bubbles, you cannot measure what happens in the fermenter accurately and you cannot use the data in an effective way to control the cellular processes. Accurate measurements in real-time are critical for the development of process protocols and for the control and maintenance of optimal process conditions. They can only be achieved by creating a bubble free measuring environment because computational approaches like averaging still lead to systematic errors.

Cytoprom has developed a bubble excluder (patent EU07705249.6) that constantly replenishes the measurement area with sample via impeller-driven fluid flow but excludes bubbles completely. Bubbles are excluded by statistical elimination through a series of baffles and by flow path engineering, which controls the flow through the device. In control experiments in which airflow rates and agitation speeds were varied no interference from bubbles into the measuring window was observed for the full range of aeration and agitation (0-80 l min^{-1} and 0-800 rpm, respectively) used (**Fig. 2**).

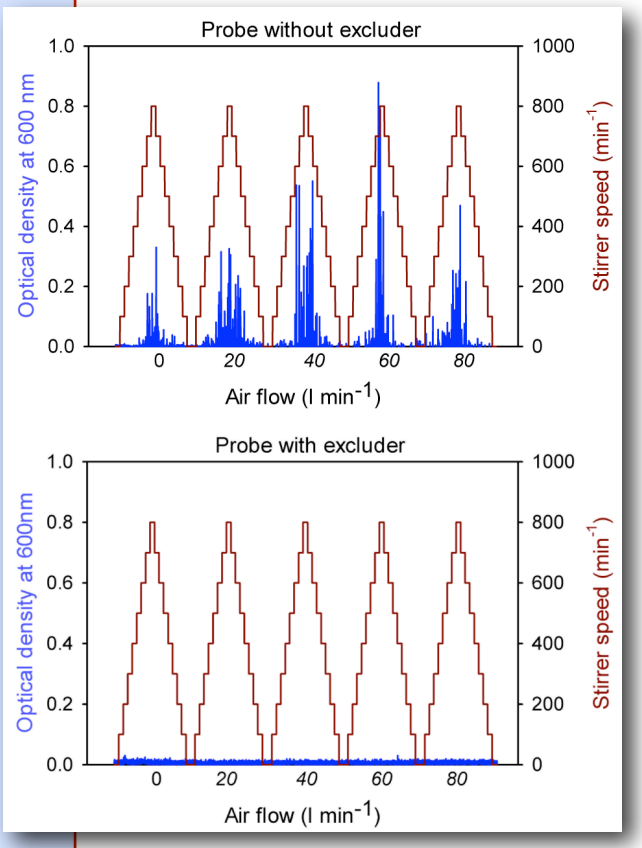

Fig. 2: For different air flowrates, the stirrer speed (red line) was varied stepwise in an Applikon ADI1075 70 L bioreactor and the optical density was measured in a salt solution (blue line). Spikes in the optical density (left panel) show interference of the measurements by air bubbles. Interference is not present at any of the conditions where the bubble excluder was used (right panel).

Growth curve measurements of yeast Yarrowia lipolytica with and without bubble exclusion demonstrate that accurate growth curves can be obtained only when bubbles are excluded from the culture (**Fig. 1**). When bubbles are excluded, the opportunities for utilising optical data are greatly enhanced. For example, on-line, in situ measurements have been used to indicate induction conditions accurately and increase expression yields. The noise-free density measurements can now be used in closed control loops to trigger additions of expression inducer via a pump when the right growth conditions have been reached. The same approach might also be applied to continuous, chemostat or turbidostat cultures where pumps are used to control feeds and product streams to maintain steady-state constants, such as the concentration of limiting substrate or biomass concentration. Automation liberates processes from working day

schedules to increase production efficiency and maximise cost savings.

In any culture with accurate growth monitoring, potential growth limitations can be easily identified. An example of a yeast strain metabolising multiple carbon substrates is given (**Fig. 3**), and the different phases of growth are clearly distinguished. It would be very difficult to discern subtle growth effects by on-line measurements with bubble interference or by off-line manual sampling. The effect on growth of the introduction of three extra histidine residues on the expressed histidine-tagged mitochondrial ADP/ATP carrier could be detected through a subtle difference in the doubling time (**Fig. 3**).

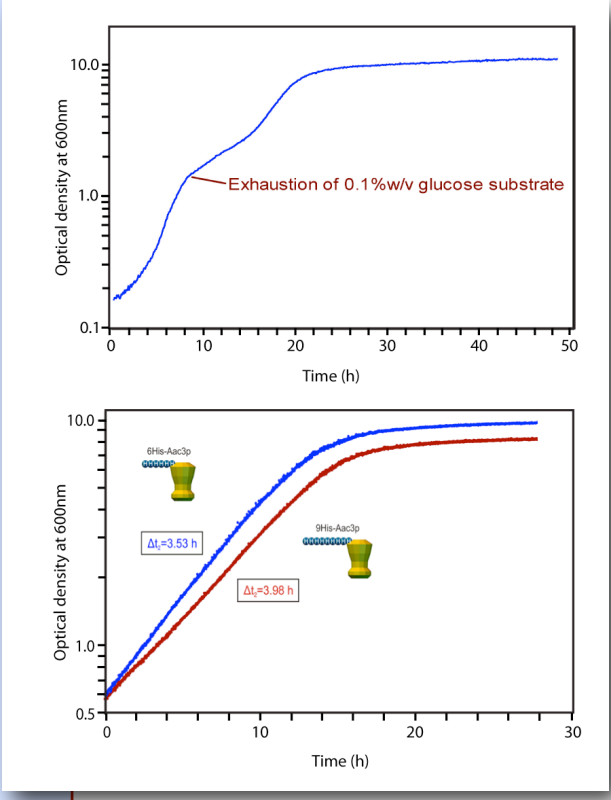

Fig. 3. With accurate monitoring, growth-limiting factors such as a substrate limitation can be easily identified in real-time and used to increase productivity. Monitoring enhanced by bubble exclusion even allows to detect a subtle change in doubling time as a consequence of the introduction of three extra histidine residues on the expressed histidine-tagged the mitochondrial ADP/ATP carrier.

Monitoring opportunities with the Cytoprom system are not limited to single wavelengths. It is now possible to obtain complete spectra without bubble interference for the best ever process information. Cytoprom offers UV-VIS systems that can measure from 215-1025 nm and NIR systems that can measure from 900-2300 nm. Complete spectral data can be collected in real-time and used to obtain profiles of processes instantly. A spectral fingerprint allows detection of cell growth and media changes, as well as a quick detection of problems, such as contaminations. Some process indicators have very weak signals, so the exclusion of bubble interference is essential if those signals are to be accurately detected.

With the patented Cytoprom system on-line measurements of cell cultures can be carried out in real-time without bubble interference across wide spectral ranges. None of the accepted benefits of optical probes are lost, and calibration and operation procedures are simple. The bubble excluding probes of Cytoprom have been shown to withstand in-place cleaning and sterilisation procedures during 4 years of continuous operation. Spectral data can be recorded as a standard 4-20 mA output signal, providing compatibility with controllers for process automation. The system components are compact and the bubble excluding probes are available in vertical and horizontal orientations to suit most fermenter types. The highly affordable Cytoprom monitoring system will pay itself back quickly through increased yields and reproducibility.

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