INFORS HT

White Paper

Integrated Biomass Measurement in Shake Flasks

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Abstract

Despite its popularity as a small-scale bioreactor, the shake flask has remained a "black box" in terms of information about the processes taking place during cultivation in an incubator shaker. The continuous, real-time measurement of biomass in shake flasks takes bioprocessing to the next level, from a "black box" cultivation system to an integrated bioprocessing solution with close environmental control and comprehensive process information from the very start.

INFORS HT offers a comprehensive and user-friendly integrated system for biomass cultivation and monitoring as a combination of INFORS HT incubator shakers with aquila biolabs online biomass measurement system CGQ (Cell Growth Quantifier) with the best price, performance and expansion capabilities. The present paper focuses on microbial bioprocesses due to the ongoing development required of the CGQ technology for classical mammalian cell culture processes.

Contents

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1 Introduction

Despite its popularity as a small-scale bioreactor, the shake flask has remained a "black box" in terms of information about the processes taking place during cultivation in an incubator shaker. While environmental parameters, such as shaking speed, temperature and humidity are closely controlled by today's incubator shakers, most users rely on manual sampling-driven approaches to monitor their cultivation. The determination of such simple yet crucial parameters as biomass or growth rate requires an interruption of the fermentation process, the removal of shake flasks for sampling and the manual evaluation of each single sample using e.g. a benchtop spectrophotometer. Overcoming this tedious, repetitive task is desirable and requires the application of appropriate technology with respect to the parameter of interest. While automated pH, pO_2/DO and CO_2 monitoring has been implemented for shaken cultures in the last decade, there has been a lack of information regarding the most important parameter in cell cultivation processes, namely the biomass or other measures that quantify the concentration or number of cells in the cultivation broth. A truly integrated solution for continuous, non-invasive, online measurement of biomass has proven elusive so far.

The integrated system supplied by INFORS HT focuses on biomass measurements and provides the means to open the "black box" and shed light on what happens at any given time point during the cultivation in a shake flask. Since biomass measurement is a key parameter for many bioprocesses, reliance on these measurements is highly advantageous.

When aiming for a specific culture density, live data indicates when a particular value is reached. Thus, cultures are in optimum condition for further steps such as utilization as seed.

On the basis of a continuous high-resolution data flow, growth kinetic parameters become accessible for the user and provide much deeper insights into the cultivation state than threshold values can do. Exponential, stationary and other growth phases, diauxic growth, oxygen limitations, inhibitory effects and even morphology changes can be easily identified. Rapid data collection and statistical analysis for comparative studies is beneficial for process optimization, media screening, toxicity studies, growth profiling, mutant selection, etc.

Integration and automation bring benefits to laboratory workflows, data analysis and validation of particular methodologies. Both industrial and academic users benefit from speed and removal of risks related to manual sampling. Consistency of outcomes leads to reductions in downtime, repeated experiments and poor outcomes.

It is vital that a system designed to work with shaking cultures uses robust, reliable technology which can accommodate a wide range of media and other growth conditions. Detectors which work on light scattering have a good combination of accuracy, range and insensitivity to variables such as media coloration. By using an integrated system, the whole is more than the sum of its parts, providing proven compatibility and skilled system support. The future of bioprocessing is trending towards more integration and automated interaction between equipment types. Providing this benefit for processes at the start of the bioprocess chain is a major step forward.

2 System requirements

Defining an integrated system for biomass measurement in shake flasks with enhanced usability and applicability involves listing the key requirements of importance to almost any user and then describing which features would provide a suitable solution. Requirements that are generally considered as important to any user are summarized and specified in Table 1.

Requirement	Description
Non-invasive measurement	No need to interrupt the cultivation process and to remove flasks from the incubator. • Avoid temperature and humidity glitches. • Avoid poor mixing and oxygen limitation.
Continuous, parallelized and real-time	Continuous and parallelized data acquisition without delays, allowing for real-time monitoring and evaluation of cell growth in multiple cultivations at the same time.
Accurate and noise tolerant	Ensure optimal accuracy for a wide range of applications. Implement proper noise treatment during data acquisition and denoising or data smoothing technologies during data visualization and analysis.
Suitable for any organism	Biomass monitoring should be possible for every organism that can be cultivated in an incubator shaker.
Suitable for any shake flask and incubation setup	The size, type, capacity and operating range of the incubator shaker within an integrated system as well as the size, type and mounting method of shake flasks should not be a barrier to using biomass measurement
Link biomass data to visualization, archiving and analysis software	Any monitoring system is only as good as the way it handles the process data generated. Basic functions such as charting, archiving and exporting should be enhanced by the ability to turn raw data into meaningful information with the help of statistical analysis and annotation.
Simple setup and calibration	As time is critical, biomass monitoring needs to be quick to set up, then easy to use on a day-to-day basis. This requirement involves physical handling, calibration and data management.
Correlation to existing methodologies	Enable users to correlate online biomass data with existing measurements, such as offline optical density at 600nm or cell dry weight.

Table 1: General requirements specification of an integrated system for biomass monitoring in shake flasks.

3 The solution from INFORS HT

Implementing the listed features into one comprehensive and user-friendly integrated system for cell cultivation and monitoring succeeds through the combination of INFORS HT incubator shakers with aquila biolabs online biomass measurement system CGQ (Cell Growth Quantifier) and provides the best price, performance and expansion capabilities. Details are as follows:

3.1 Non-invasive measurement

The biomass monitoring relies on a sensor array mounted on a plate which is placed beneath the shake flask. As depicted in Figure 1, LEDs irradiate light through the shake flask bottom into the culture and the light that is backscattered by the cells is measured by a detector array very rapidly and continuously. This backscatter signal increases with biomass and provides non-invasive high-resolution growth curves (Figure 3) avoids negative side effects from stopping and restarting the incubator shaker, e.g. temperature or humidity glitches, poor mixing and transient oxygen limitation.

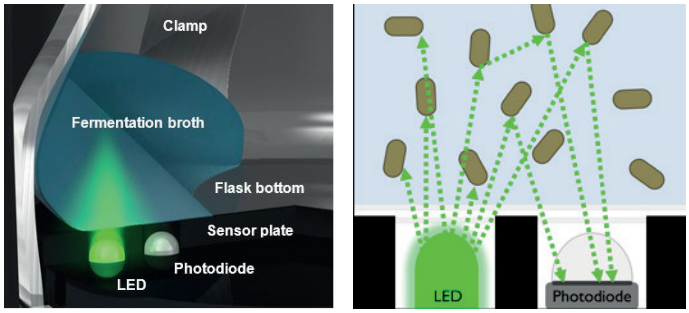

Figure1: Biomass monitoring by backscattered light detection – the CGQ sensing architecture.

3.2 Continuous, parallel and real-time

Biomass data is acquired continuously on each sensor plate with a data density that allows for real-time monitoring of each single flask. This provides a resolution and quality of data which is invaluable to any applications related to kinetic growth studies. Data streams from multiple sensor plates are bundled at a central base station and forwarded to a local computer via USB. Up to 16 sensor plates can be operated from one base station, allowing for unprecedented parallelization in real-time online monitoring of shake flask cultivations. With its integrated system for biomass measurement and cultivation, INFORS HT enables users for the first time to monitor and extract knowledge from each single flask in their incubator shaker.

3.3 Accurate and noise tolerant

The backscattered light sensor is designed for optimal accuracy in a continuously shaken environment. Its unique data acquisition technique and data processing algorithms work on subsampling at a microsecond level and can extract data from shaking induced intensity fluctuations, which impairs other state-of-the-art sensors by

100 mL sensor plate 250 mL universal adapter sensor plate fpr 250 mL flask

Figure 2: One CGQ sensor plate monitors each kind of shake flasks using a a suitable universal adapter.

appearing as shaking noise. In order to achieve maximum sensitivity, a darkening cover placed over the shake flasks prevents the influence of ambient light on backscatter measurements. For extremely noisy environments, such as heavily shaken baffled flasks, the CGQuant software provides tools for data smoothing and noise rejection.

3.4 Suitable for any organism

The INFORS HT integrated biomass monitoring system is capable of providing accurate results for a wide range of bacteria, yeast and fungi in many different growth media. Table 2 enlists organisms that have been successfully monitored. Specific information regarding media, organisms and exemplary growth curves is available on request.

Cell type	Organisms
Bacteria	*Escherichia coli, Corynebacterium glutamicum, Bacillus subtilis, Gluconobacter oxydans, Pseudomonas putida, Streptomyces venezuelae, Vibrio natriegens, Vibrio cholerae, Staphylococcus aureus, Klebsiella pneumoniae, Lactobacillus plantarum, Actinobacillus pleuropneumoniae, Chromobacterium violaceum, Acetobacterium woodii, Clostridium acetobutylicum, Clostridium ljungdahlii, Prevotella copri, etc.*
Archaea	*Sulfolobus acidocaldarius, Haloferax volcanii*
Fungi / Yeast	*Saccharomyces cerevisiae, Schizosaccharomyces pombe, Pichia pastoris, Yarrowia lipolytica, Kluyveromyces lactis, Hansenula polymorpha, Aspergillus niger, Ustilago maydis*
Algae / Plant Cells	*Chlorella vulgaris, Nicotina tabacum BY-2*

Table 2: Organisms that have been cultivated using the INFORS HT integrated biomass monitoring system.

3.5 Suitable for any shake flask and incubation setup

Using one type of sensor plate and a set of universal adapters (see Figure 2), the biomass monitoring system can be supplied for flask sizes from 100 ml to 5000 ml total volume, made from either glass or single-use plastics. Standard stainless steel clamps or the INFORS HT green "Sticky Stuff" can be used for flask attachment as required. The biomass sensor has been optimized for high oxygenation conditions with filling volumes of 5 - 15 %. However, even lower filling levels and also filling volumes above 20 % can be monitored with some trade-offs regarding sensitivity. In combination with stainless steel clamps, the full range of shaking speeds is available for biomass monitoring. Applications with an inherent need for sampling benefit from shaking synchronization, which automatically pauses the biomass measurement as soon as the incubator shaker is opened.

3.6 Link biomass data to visualization, archiving and analysis software

The biomass data from single or multiple flasks is relayed from the base station in the shaking incubator to a Windows computer running CGQuant. The central software provides features regarding measurement control, real-time charting, data comparison, statistical analysis of replicates, archiving, annotation and export of data. Users of the INFORS HT integrated biomass monitoring system get access to a toolset that enables efficient process control, identification of relevant process events, detailed data analysis and rationalized knowledge generation as required in modern Design-of-Experiment processes.

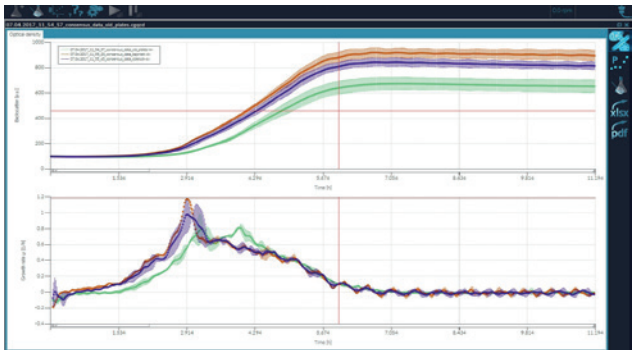

Figure 3: CGQuant and CGQ as biomass sensor component in the integrated system for biomass monitoring in shake flask cultures from INFORS HT.

3.7 Simple setup and calibration

The physical set up in the incubator shaker is quick and easy. Once mounted, the sensor plates can be used with different flask sizes by simply exchanging the adaptor plates. The hub system is prepared for linking up to 8 sensor plates, with an alternative hub capable of increasing this to 16. A cable connection is made from each sensor plate to the hub and one from the hub to the CGQuant computer. The software then handles experiment configuration, data visualization and annotation. Data can be exported as image files or as complete reports in the XLSX or PDF format for further usage and archivin. Calibration uses the familiar technique of creating a standard curve and the whole process is guided by the software. Calibration data can be stored for re-use and previously collected datasets can be post-calibrated with newly generated calibration files.

3.8 Correlation to existing methodologies

Direct comparison to offline optical density measurements in an external spectrophotometer is good and the software is specifically tailored to collect calibration data that allow for the calculation of offline biomass measures from the backscatter data. Of course, the practical problems inherent to external manual measurements must be taken into account. Different organisms, media and fluid dynamic conditions require separate calibration files, due to their influence on the backscatter efficiency and light distribution in the shake flask.

4 Summary

The continuous real-time measurement of biomass in shake flasks takes bioprocessing to the next level, from a "black box" cultivation system to an integrated bioprocessing solution with close environmental control and comprehensive process information from the very start. The inconvenient and inherently interventionist approach used to date with manual sampling can, at best, only provide a few clues as to what is happening during cultivation. This would not be acceptable for any other process value such as temperature or shaking speed, so why accept it as inevitable for biomass? Of course, the measurement system used must be an integrated part of the complete system while providing simplicity, economy and validity.

All the generic key requirements for such a system can be met with the INFORS HT integrated biomass monitoring. The widest possible range of organisms, media, volumes and flask types are catered for with a non-invasive, reliable and effortless measurement technology based on light backscattering. Closely controllable environmental parameters in the incubator shaker work hand in hand with a biomass measurement technique that provides growth data under almost any cultivation condition, even in the noisy environment of a heavily agitated shake flask. A fully automated approach to experiment setup, calibration, data handling and comparison of multiple inputs provides the means to turn raw data into usable process information and allows for a deeper bioprocess understanding.

Applications for biomass monitoring at this level fall into several broad categories, amongst others:

- Optimization of growth over time, reducing time or amount of experiments

- Ending reliance on the quality of manual methodologies
- Reaching a target value for biomass and having an indication when this happens
- Comparing growth kinetics to identify biopro cess events that remained invisible at the past times of sporadic manual sampling and biomass determination.
- Comparing media compositions to maximize growth
- Toxicity screening
- Mutant screening and growth profiling
- Validation of the biomass in a seed culture without compromising sterility, while allowing comparison to previous batches to indicate excursion in the cultivation process.

The trend in all areas of research and production goes towards distinct control of bioprocesses and a quicker response to changes within the process with clear evidence of what is happening during cultivation. Biomass monitoring brings these values to the standard laboratory shake flask.

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