INFORS HT

White Paper

Continuous Culture as the Universal Bioprocess for Manufacturing

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Abstract

In recent times, continuous culture has moved from a niche cultivation technique to a way of allowing better automation and control in industrial bioprocesses. Things are at an early stage but the potential of continuous culture to become a "universal bioprocess" over time is clear to see. Continuous culture is useful when you have a genetically stable population of microorganisms and a product which can be expressed at a constant rate for a long time without altering. It allows the maximum productivity per batch and reduces the overall time needed for cleaning, sterilisation and handling of the vessel. The various methodologies, equipment and control strategies are described, along with specific reference to prepared solutions which are available from INFORS HT.

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1 Introduction

Microbial and cell culture bioprocesses can be performed in several ways in terms of additions and removal of liquids. Batch, fed-batch and perfusion are the commonly used. Continuous culture is a special case where medium is both added and removed for much of the fermentation time, in ways which keeps cell numbers in balance.

This concept has been advocated for some time e.g. Rathore et al (2016) and posits that there are significant economic, flexibility and automation benefits to be made from applying continuous culture methodologies to certain bioprocess applications such as production of biosimilars in mammalian cell culture (Langer & Rader, 2014). Continuous culture has been unfashionable for some decades, apart from the few research groups doing metabolic studies or making some "proof of concept" studies for process development on a small scale. The idea of making a scalable, continuous process for industrial production has only been revived in recent times, the main driver being the use of cell culture for monoclonal antibodies or therapeutic protein production. This has led to a re-think of the value of continuous culture generally as a production technique which provides cost savings and the change for a high level of automation.

Bioprocess equipment design comes into the story with dedicated packages for continuous culture and perfusion, as any new process would first have to be thoroughly tested at the laboratory scale. New methodologies, control strategies and integration of downstream processing can all be developed before any commitment to scale up is made. In fact, for small-volume, high-yield processes, the scale could be quite modest (10's of liters) as the productivity of a continuous process can be high, e.g., monoclonal antibodies at a concentration >1 g L 1. Bioprocess software scalable for both research and production environments is also critical, including the ability to add metadata related to quality control and validation. Access to this information remotely for each type of user is essential.

Movement in this direction will not happen immediately and will not be done in a single step. Development of such processes on a small scale for academic and industrial research would have to be amenable to use of soft sensors for novel solutions to whole bioprocess control, integration of third-party hardware and software, and planning of experiments on a potentially very large scale.

2 Why use continuous culture?

The value of continuous culture as a methodology stems from the basic idea that when conditions are held constant, individual influences on the growth and metabolism of cells can be better investigated. Also, for a production environment, it provides a process state that is amenable to control and evaluation techniques difficult to perform where dynamic phases are involved. Given these two complimentary uses, the applications in research and industry and the benefits using continuous culture can be summarized as follows:

Research:

- Investigations in cell biology, as a source for large volumes of uniform cells or protein
- Enrichment for specific types of bacterial mutants, such as auxotrophs, or those that are resistant to antibiotics or bacteriophages for further scientific study
- Collecting steady state data about an organism to generate a mathematical model relating to its metabolic processes
- Studying the metabolic strategies pursued by the organisms at different growth rates by varying the dilution rate
- Analyzing metabolic pathways in conditions of starvation, optimum concentration and excess of a growth-limiting substance

Industry:

- Frequently used in the industrial manufacturing of ethanol
- Development of experimental models of continuous cultures in the biotechnological industry for maximizing equipment use, minimizing downtime and providing culture conditions well suited to statistical process control methodology with comparison to models.

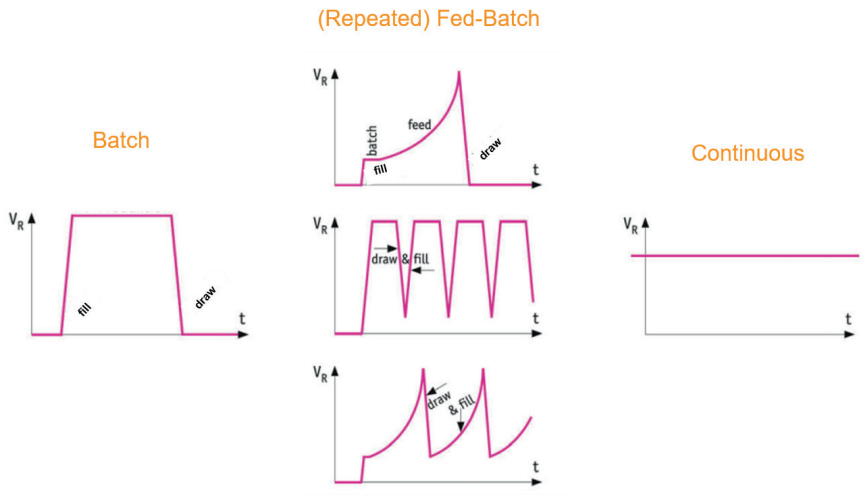

Figure 1. Schematic depiction of the bioreactor volumes during Batch, (Repeated) Fed-Batch and Continuous bioprocesses. (Based on H. Chmiel, Bioprozesstechnik, 2006)

Advantages:

- Media exchange: by exchanging the media continuously the cells are always supplied with fresh nutrients. Moreover, the product is harvested from the beginning on and by-products as well as toxins are steadily removed. In addition, the media exchange allows to mitigate the effects of changes in cell metabolism correlated to a declining yield of biologically-active product over time e.g. mis-folding during glycosylation.
- Increase space-time-yield: The time in growth / production phase is prolonged from days to weeks or even months.
- Achieve physiological steady state: All culture parameters and the metabolic status of the microorganism remain constant (μ, V, DO, cX, cS, cP, pH, …)
- Smaller bioreactors can be used for same amount of product, which can have a big impact for economical use of space, operator time and support services.
- The efficient use of downstream processing equipment: By dealing with smaller volumes of culture over a longer time, the capacity of such equipment can be extended by not overloading any one particular "bottleneck" in the processing pathway.
- Perfusion: Slow-growing cells are kept in the vessel, reducing the resources needed for seed production. Additionally, the downstream vessel effluent is cell-free and so more quickly processed using techniques such as colum-chromatography as a separation step can be skipped.

3 Adaptions for continuous culture

All major categories of microorganisms and cells in liquid culture are amenable to growth using continuous culture. Some methods may be more appropriate than others, according to the application, but the same general criteria hold true. Specific adaptions that are commonly made for continuous culture processes are summarized in table 1.

4 The solution from INFORS HT

The solutions offered by INFORS HT for continuous culture make use "out of the box" a reality. Starting with the Minifors 2, the bioreactors come prepared for common bioprocess strategies like continuous culture. The Minifors 2 provides four re-allocatable pumps so feed and harvest are available with the click of a button. The precise control of feed rates makes it possible to run for prolonged periods with the reassurance that the deliveries should remain accurate and so minimize issues such as wash-out. With kits providing all the necessary "plumbing" in terms of tubing and connectors, the hunt for the right size of connectors etc. is never an issue.

Adaption	Description
Peripheral Equipment	For continuous culture special peripheral equipment is needed e.g. bottles, connectors etc. Support for the right set up via documentation.
Pump for harvest	Additional pump or possibility to reassign integrated pumps, e.g., antifoam pump can be configured as a harvest pump out of the box.
Small vessel sizes	Small vessel sizes are of advantage, so working volumes can be kept to a minimum, especially for multiple, parallel experiments.
Online biomass measurement	Addition of sensors for real-time measurement of biomass using e.g. turbidity for maintaining a constant cell density over prolonged periods (e.g. turbidostat operation).
Spin filter	Use of cell separation devices such as spin filters for Perfusion techniques to keep cells within the vessel while removing cell-free supernatant and replacing it with fresh medium.
Parallel and multi-modal	Open loop control of the shake flask allows for parallelization of the cultures and, depending on the culture outcome, real-time adjustments with simultaneous data collection.

Table 1. Overview of specific adaptions that are commonly used to perform continuous culture and their description.

4.1 Peripheral equipment

All necessary peripheral equipment like bottles and connectors is available as part of a package. This may include external sampling from the effluent stream and aseptic collection of harvest for downstream processing. INFORS HT offers sets of the necessary hardware for continuous culture. In addition, a presentation, manuals for set up and training are available to support setting up a reliable, well-configured system.

4.2 Pump for harvest

Assignment of integrated pumps for feed and harvest for use of standard systems "out of the box". The antifoam pump can be configured as a harvest pump. Of course, customized vessels with other solutions for effluent removal and the use of external pumps are always possible. Additionally, eve® can be used for advanced feed control and the continuous monitoring of flows and filling levels.

4.3 Small vessel sizes

Small vessel sizes allow for working volumes to be kept as low as possible. This is especially useful for multiple, parallel experiments for bioprocess development and optimization. With 1 – 3 turnovers of vessel contents per day for a typical continuous culture, even a few hundred mililiters working volume can lead to many 10's od liters of fresh medium being consumed over 30 – 90 days. With the Multifors 2 uses the samse sensor technology as as bioreactors with larger working volumes, the risk of differences during subsequent scale-up is minimized compared to other systems.

4.4 Gravimetric feeding

The accuracy of the in and out flow rates can be improved even further using gravimetric feeding, enabling true continuous operation with a stable culture environment maintained by the addition rate of single, growth-limiting substrate. External balances can be connected to INFORS HT bioreactors and set up gravimetric feeding locally or with eve for advanced feeding functions compared to configuration on the HMI.

4.5 Online biomass measurement

Addition of sensors for real-time measurement of biomass using e.g. turbidity to maintain a constant cell density over prolonged periods. Turbidity sensors from Optek with different optical pathlengths can be directly connected to the controller and the measuring values are displayed on the touchscreen. The biomass concentration can be kept constant inside the system e.g. with feed control based on the biomass concentration via the bioprocess platform software eve®.

4.6 Spin Filter

Use of cell separation devices such as spin filters allow perfusion cultures to be performed where the cells are kept within the vessel while cell-free supernatant is removed and replaced with fresh medium. INFORS HT offers spin filters with a mesh size of 10 µm for Multifors 2 and Labfors 5 for perfusion processes with a working volume as low as 180 mL. This way essential process data can be generated with low media consumption prior to the scale-up. Spin filter are also often used in combination with microcarriers.

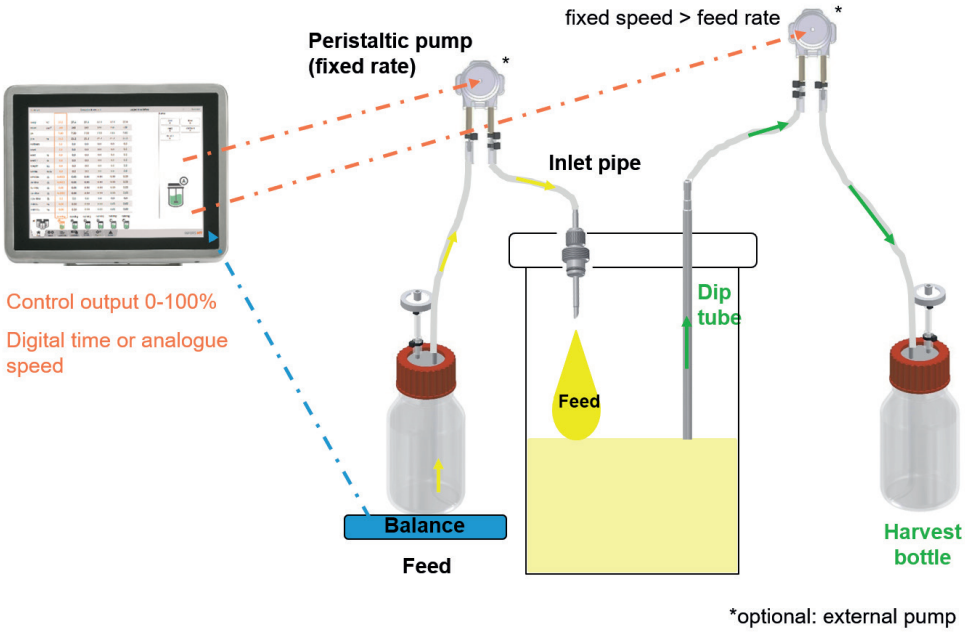

Figure 2. Schematic overview of continuous culture "out of the box".

Besides the spin filter, external devices such as an ATF (Alternating Tangential Flow) filter can be used for cell retention.

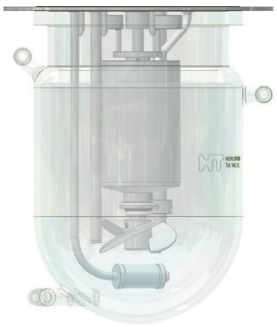

Figure 3. INFORS HT Spin Filter

4.7 Soft sensors related to continuous culture

Soft sensors can give valuable insights for continuous culture bioprocesses and help to improve feeding strategies, to calculate the dilution rate or to monitor the full/empty status of bottles. Additionally, a flexible scripting engine enables the generation and use of highly-customized, application-specific or even model-based control and data interpretation strategies.

5 Summary

The solutions provided by INFORS HT make the advantages of continuous culture available to small-scale researchers and process development laboratories as several "out of the box" solutions. Continuous cultivations are catered for, along with in-vessel separation of cells and effluent using perfusion options. The addition of sophisticated process control strategies such as gravimetric feeding via the eve® bioprocess software platform make for a complete package.

Continuous culture has been used in specific applications for decades, either for metabolic studies or optimization of resources for low value products. The advent of pharmaceutical applications for production of high-value, low-volume biologically active proteins, e.g., monoclonal antibodies, has given the opportunity for continuous culture to fulfill a new role. Production of biosimilars in mammalian cells is now a very relevant application here, as very significant cost savings Investigation of the methodologies and process optimization is the first part of this process and the packages available from INFORS HT provide the ideal toolkit for making that first step.

References:

Langer E.S. & Arder, R.A. (2014) Continuous Bioprocessing and Perfusion: Wider Adoption Coming as Bioprocessing Matures Spring 2014 BioProcessing Journal 50-54.

A. Rathore et al., Continuous Processing for the Production of Biopharmaceuticals, BioPharm International 29 (4) 2016.

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