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Large-Scale Perfusion and Concentrated Fed-Batch Operation of Biostat STR® Single-Use Bioreactor

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Introduction

Perfusion is a well known production approach since many years for a number of biological drugs such as coagulation factors (Factor VIII: Kogenate, Bayer and Refacto, Pfizer; Factor VII: Novoseven, Novo Nordisk), Protein C (Xigris, Eli Lilly) and other enzymes but also monoclonal antibodies (ReoPro and Remicade, both Janssen Biotech; Simulect, Novartis, etc.). Recently, perfusion has been used also to generate high cell density seed cultures to reduce the number of seed steps necessary to reach final production scale [1, 2].

Through the higher cell densities and titers achieved in concentrated fed-batch and perfusion cultures typically smaller culture volumes and bioreactor scales are required to produce the therapeutic protein or antibody. This is a great advantage as the footprint of a production facility can be reduced and scale-up issues are mitigated due to the marginal scale-up factor between clinical and commercial production. Intensified cell culture processes are especially beneficial in the context of single- use facilities as they provide production capacities at 1000 L scale that in the past where only achievable with five to ten times larger bioreactors [3, 4]. Furthermore, modern high-end cell culture processes aim to maintain the cells in a defined metabolic state in order to ensure stable product quality through controlling protein folding and glycosylation. In this case, the main aim is not necessarily to reach very high cell densities, but to ensure a steady state of nutrients and metabolites in the bioreactor.

Find out more: www.sartorius.com/en/products/fermentation-bioreactors/single-use-bioreactors/biostat-str

How to Perform Concentrated Fed-Batch or Perfusion Operation

After inoculation of the bioreactor and an initial 1 – 2 day batch growth phase, the removal of cell free supernatant e.g. with the Refine ATF system is started at a constant harvest flow rate. At the same time, the culture is replenished with fresh medium.

A number of alternative cell retention devices are available such as continuous centrifuges (Centritech Cell from Pneumatic Scale Angelus or kSep from KBI), internal and external spinfilters, settlers, hollow fiber and other membrane based retention systems.

When applying single-use bioreactors such as the Biostat STR®, the addition is controlled via a feed pump that receives a signal from load cells or a platform balance maintaining a defined bioreactor weight. As the cell density grows and the nutrient consumption and metabolite formation increases, the harvest rate is subsequently increased to maintain a certain exchange rate of fresh medium per cell or alternatively a given medium exchange rate per day [2]. On-line biomass measurement, e.g. with the BioPAT® Viamass probe that will soon be available for single-use Flexsafe STR® and RM bags, provides an automated option to control the perfusion rate based on cell density. Using at-line glucose and lactate measurement, e.g. with the BioPAT® Trace, an additional concentrated feed can be applied to control the glucose concentration.

Figure 1 provides a schematic depiction of a typical concentrated perfusion or fed-batch set-up based on the Biostat STR®.

Key Considerations

Typical perfusion rates are in the range of 1 – 2 bioreactor volumes per day. Applying a small cell bleed stream enables the establishment of a defined cell growth rate and by that a high viability can be maintained which in turn mitigates clogging of the cell retention device [5]. Dependent on the pore size or cut-off of the cell retention membrane, either the product is recovered in the cell free harvest (concentrated perfusion) or in the bioreactor content (concentrated fed-batch). As most antibodies are rather stable, concentrated fed-batch with accumulation of the product in the bioreactor is a simple and straight forward approach to increase space time yields of a given facility. Concentrated perfusion is the method of choice for recombinant proteins that in many cases are prone to degradation or might show feedback inhibition and should therefore be removed from the cell culture into a chilled harvest tank and subsequently purified.

Media Logistics

In large-scale continuous processing, media and harvest logistics needs specific attention. Dependent on the bioreactor scale and chosen perfusion rate, 500 L to 2000 L and more fresh medium have to be provided daily. The Flexact® MP system is an easy to use single-use system for automated media preparation (Fig. 2) especially designed to reduce effort and increase convenience associated with large-scale continuous operation. Its design encompasses dust free transfer of powder media into a mixing bag, a single use sensor for pH measurement and adjustment and a controller that manages automatic sterile filtration and transfer into a storage bag. In case of perfusion, equal volumes have to be collected and processed in subsequent purification steps. Our range of chilled Palletanks® provide a proven single-use option for storage of large harvest volumes in disposable bags. In summary, the Flexact® MP system together with Palletanks® and Flexel® bags manage the challenging fluid handling of large scale, singleuse continuous processing.

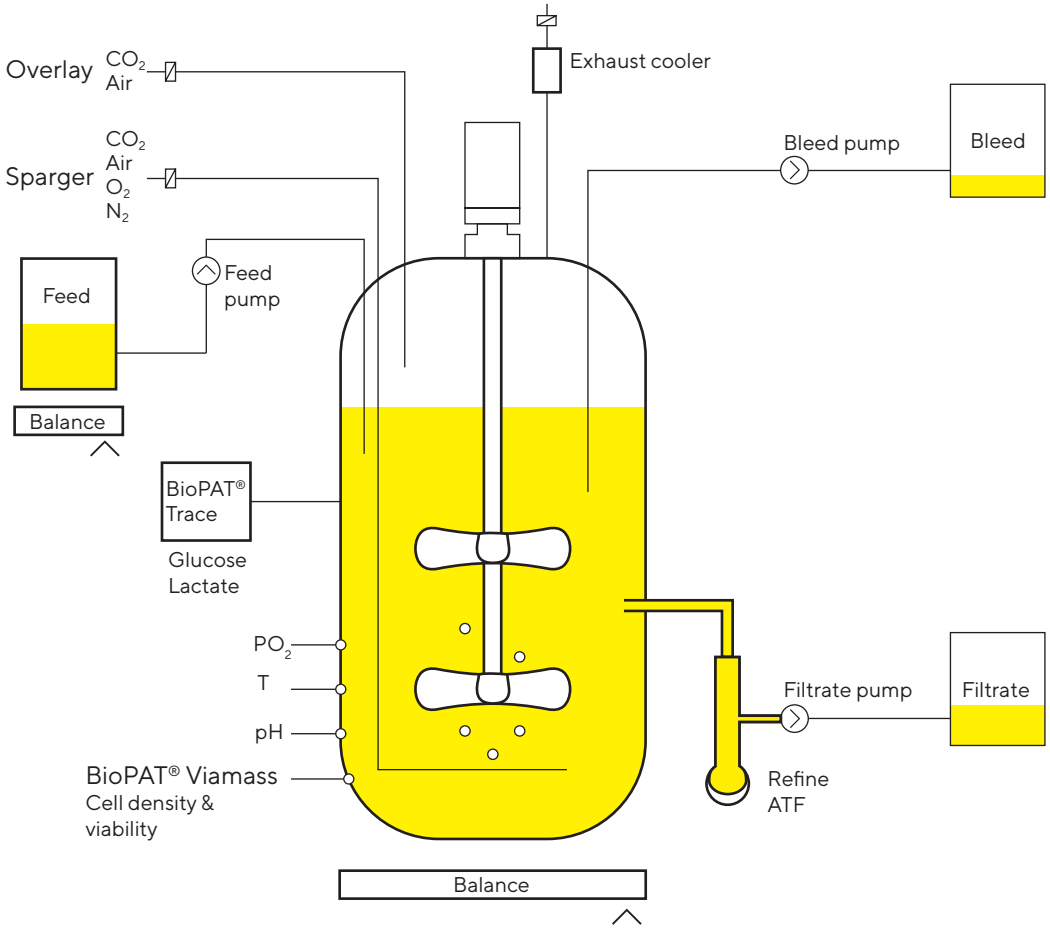

Fig. 1: Set-up of a concentrated fed-batch using the single-use Bioreactor Biostat STR®.

Fig. 2: Flexact® MP – Disposable solution for media preparation.

Scaling up Continuous Processing

Concentrated fed-batch and perfusion processes can be developed and successfully implemented at production scale using Sartorius stirred tank single-use bioreactors in combination with different sizes of the Refine ATF module. At the 2L bench scale, e.g. our Univessel® in combination with our Biostat® B or B-DCU controller provides a fully scalable development system. Subsequent scale-up from 50L and 200L can be achieved to the 1000L scale in the Biostat STR®. At large-scale, the ATF modules might be connected via side ports of the single-use bioreactor bag using up to two 1" sterile connectors and operated in an external loop of the bioreactor (Fig. 7). It is critical that this external loop is as short as possible to avoid that the cell culture is exposed to uncontrolled conditions, e.g. different temperature and potential oxygen limitations.

Single-Use Bioreactor Configurations Suitable for Intensified Cell Cultures

Key to successful concentrated fed-batch and perfusion operation is an efficient aeration system that provides kla values above 10 – 15 h^{-1} to supply the culture with sufficient oxygen (Fig. 3). At the same time, excessive CO_2 is formed in the intensified culture which needs to be removed to avoid any inhibitory effect on productivity or even product quality.

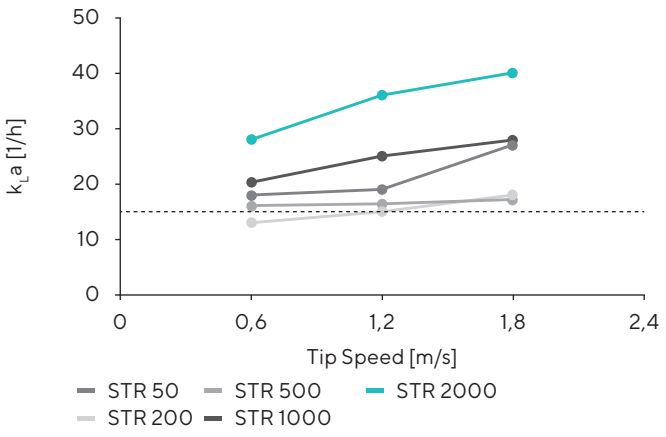

Fig. 3: kla values determined in different single-use bioreactor bag volumes of the Biostat STR®, equipped with 2 × 3blade segment impellers, using the gassing out method in phosphate buffered saline, aeration rate 0.1 vvm, 150 µm holes of combisparger, temperature 25°C.

This can be achieved with the Combisparger that microsparges compressed air or pure oxygen through defined 150 µm holes and provides a stripping gas flow through 0.8 mm holes at the same time (Fig. 4). This single-use sparger design emulates a successful aeration strategy applied since many years in conventional stainless steel bioreactors.

Fig. 4: Combisparger with 150 µm defined micro-holes and 0.8 mm holes.

A problem that should not be underestimated is excessive aerosol formation in the exhaust gas due to high gas flow rates and high protein content in the concentrated cell cultures. A specifically developed single-use exhaust cooler design based on the well-known principle of plate heat exchangers (Fig. 5) mitigates the risk of blocked filters and increases process reliability dramatically [6]. Additional safety locks in the bioreactor control software prevent bioreactor overflow in case of clogging of the cell retention device. As a worst case safety lock, all feed pumps and gas flows are interrupted if the pressure in the bioreactor exceeds the maximum defined operating pressure.

Fig. 5: Exhaust Cooler

Conclusion

Modern single-use bioreactor designs such as of the Biostat STR® allow advanced, intensified cultivation strategies whilst providing tools to mitigate operational risks associated to a complex bioprocessing strategy and thus enabling robust single-use production for clinical trials and commercial drug manufacturing.

References

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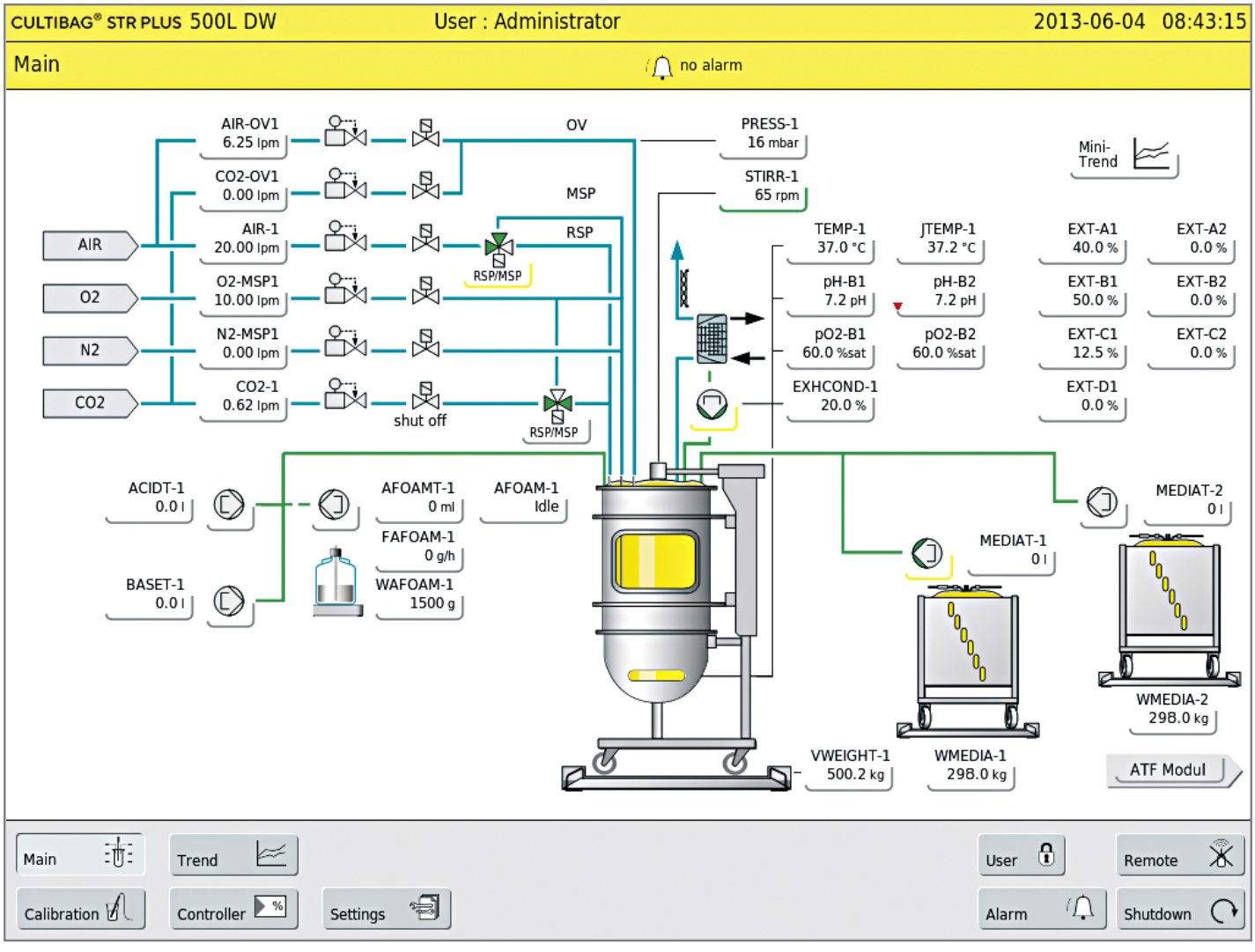

Fig. 6: Screenshot of Biostat STR® controller configured for perfusion operation using ATF module.

Fig. 7: Biostat STR® 200L with connected Refine ATF system

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