

INTEGRATION OF LOW-pH VIRAL INACTIVATION AND PRIMARY CLARIFICATION PROCESSES IN A SINGLE USE DISPOSABLE BIOSETTLER

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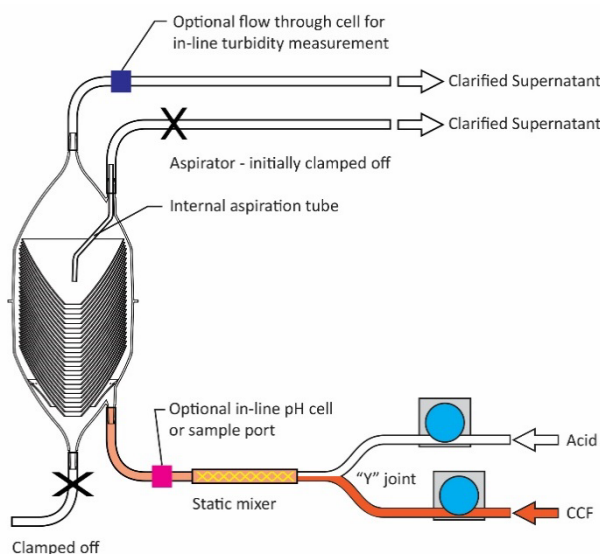
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Enhanced sedimentation on inclined surfaces has been exploited successfully in mammalian cell cultures to recycle live and productive mammalian cells into the continuous perfusion bioreactors, while removing selectively the smaller dead cells and cell debris into the harvest stream. We have developed a compact settler design using cylindrical and conical geometries, with about 5-10X more settling surfaces over a given footprint, compared to the traditional rectilinear scale up design.

We have recently fabricated the compact settler as a single use disposable plastic settler at an initial size of 150 mm diameter ("BioSettler150"). Continuous perfusion bioreactors attached with this BioSettler as the selective cell retention device achieve high cell densities and viabilities of CHO cells over extended culture durations by selectively removing smaller dead cells and cell debris. We have now successfully demonstrated our BioSettler to clarify cell culture broth from the end of fed-batch culture, recovering over 95% of secreted antibody product in the clarified harvest stream and reducing the turbidity of cell culture broth by ~ 80% in clarified harvest stream. This primary clarification using the single use disposable BioSettler is a very gentle process, without any measurable increase in cell lysis (or LDH activity).

Our clarification process is accelerated by faster settling of cell clumps, formed due to acidification of cell culture broth to a pH value of 5 or less, which can be carried out within the bioreactor at the end of fed-batch culture or in the BioSettler. If the pH is lowered to 3.5 for the requisite period of time within the bioreactor or its adjacent BioSettler, our cell aggregation and clarification process can replace a later low-pH viral inactivation step, moving it closer to where any adventitious viral replication may occur. An adjacent secondary clarification step by depth filtration can replace the need for a later viral clearance filtration step.

During continuous flow clarification



The BioSettler has been scaled down to 250 ml volume for gentle concentration and harvesting of induced pluripotent stem cell clusters and up to 25 liters so far for many larger pilot-scale applications. such as (i) clarification of the cell culture broth, (ii) precipitation of antibody aggregates, (iii) selective retention of live cells, (iv) separation of products in organic phase from aqueous fermentation broth, etc.