

Biotechnology Congress 2015 : Chemically modified cysteine in fed-batch processes and impact on CHO specific productivity - Aline Zimmer - Merck Millipore

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Industrial fed-batch cultivation of mammalian cells is used for the production of therapeutic proteins such as monoclonal antibodies. Beside medium ensuring initial growth, feeding is necessary to improve growth, viability and antibody production. Established commercial systems include a slight acidic concentrated main feed and a separate alkaline amino acid feed containing L-tyrosine and L-cysteine. Since L-cysteine is not stable due to its dimerization to cysteine in the presence of air and metal catalysts, a stable L-cysteine derivative is needed to include all amino acids in neutral pH feeds. Those single feed systems are favored to simplify feeding schemes and improve the overall process robustness through stabilization of both pH and DO signals. Here, we suggest the use of a chemically modified cysteine in combination with phosphor tyrosine disodium salt in an industrial single feed fed-batch process applicable at small scale as well as in bioreactors. Cell culture experiments were carried out either in spin tubes or bioreactors with a CHO suspension cell line expressing a human monoclonal antibody. Viable cell density and viability were measured using an automatic cell counting device. Spent media analysis of supernatants was carried out for amino acids after pre-column derivatization and UPLC analysis and for vitamins using LC-MS/MS.

Metabolite measurements were performed with Cedex Bio HT relying on photometric and turbidometric methods. Characterization of the monoclonal antibody was performed using 2-AB labeling for glycan analyses, cIEF for charge variants analyses and LC-MS/MS for peptide mapping experiments. Stability studies of the feed containing the modified cysteine derivative showed that the molecule was stable and that no L-cysteine or L-cystine was released over three months when stored

at room temperature or 4 °C. Moreover, no change in the color of the feed was observed over time. Small scale batch experiments where L-cysteine was replaced by the same amount of chemically modified cysteine indicated no change in growth or viability profiles. Use of the modified cysteine derivative in small scale fed-batch processes indicated comparable maximum viable cell density, prolonged viability and increased titer compared to the established two feed system.

Bioreactor experiments confirmed the increase in specific productivity described at small scale when the single feed strategy was compared to the two feed strategy. In depth characterization of the monoclonal antibody indicated no change in the glycosylation or charge variant pattern whereas peptide mapping experiments were not able to detect any integration of the modified amino acid in the sequence of the monoclonal antibody. Mammalian took care of clump forms for monoclonal neutralizer (mAb) creation depend on vital taking care of a few supplements, for example, glucose, nutrients and amino acids to expand culture time and improve protein production[1]. In real procedures, L-cysteine and L-tyrosine are taken care of independently at soluble pH due their low steadiness and low solvency at impartial pH, bringing about pH tops and precipitations[2]. To improve cutting edge forms, both amino acids have been artificially altered to upgrade their particular solidness and dissolvability profiles. Past work has exhibited that phosphotyrosine disodium salt (PTyr2Na) is a steady L-tyrosine subordinate and can be utilized in unbiased pH takes care of without detectably affecting the way of life execution or the mAb quality attributes[3]. Here, we present outcomes got utilizing a L-cysteine subsidiary in a nonpartisan pH, single-feed framework.

The dependability of the L-cysteine subsidiary was assessed in nonpartisan pH, Cellvento™ Feed-220 during a quarter of a year at room temperature or 4°C. For took care of group societies, a CHO K1 clone communicating a human mAb was utilized. Development was acted in the Cellvento™ CHO-220 media framework as indicated by the item procedure direction. For controls, Cellvento™ Feed-220 and a different basic cysteine/tyrosine feed were utilized though in the single feed process, the L-cysteine subsidiary and PTyr2Na were legitimately solubilized in the unbiased pH, primary feed. Trials were acted in turn tubes and 1.2L bioreactors. Development and reasonability were observed utilizing a ViCell®, titer was resolved utilizing Cedex Bio HT and explicit profitability was determined dependent on titer, vital feasible cell thickness and weakening.

For spent media investigation, the L-cysteine subsidiary and amino corrosive measurement was performed by UPLC using AccQ-Tag™ Ultra Reagent. To evaluate the responsive capability of the feed, H2DCFDA was added to the nonpartisan pH, Cellvento™ Feed-220 enhanced or not with the subordinate. For intracellular responsive species evaluation, cells were marked with carboxy-H2DCFDA and fluorescence was estimated. To assess the limit of the cells to use the subsidiary, cell lysates were spiked and shaped items were evaluated by UPLC. For mAb examination, N-glycosylation was measured utilizing HPLC after 2-AB marking, while charge variations were resolved utilizing cIEF.

Investigation of the L-cysteine subsidiary steadiness in impartial pH feed demonstrated no adjustment in the subordinate fixation nor L-cysteine/L-cystine discharge when put away more than a quarter of a year at room temperature or 4°C. No precipitation or shading change was watched showing that the subordinate was steady under the tried conditions. Taken care of bunch development in turn tubes with the single-feed procedure brought about higher last viabilities prompting upgraded last titers when contrasted with the control condition. Turn tube

results were affirmed in bioreactors prompting higher last viabilities, expanded titers and higher explicit profitability with the single feed methodology.

Lower color oxidation was estimated after expansion of H2DCFDA to unbiased pH, Cellvento™ Feed-220 containing the subordinate contrasted with feed alone showing lower receptive potential. Lower intracellular color oxidation was identified by expansion of carboxy-H2DCFDA to turn tube single-feed took care of bunch societies showing lower intracellular receptive species age when utilizing the subordinate. At the point when spiked into cell lysates, the cysteine subordinate was used to cysteine. No distinction in N-glycosylation or charge variation was recognized in the delivered mAb demonstrating no impact of the subsidiary on the introduced basic quality characteristics. This examination shows that the L-cysteine subsidiary and PTyr2Na can be incorporated into a nonpartisan pH take care of and can be utilized as a wellspring of L-cysteine in took care of cluster forms prompting a higher explicit efficiency contrasted with the best in class process without influencing mAb basic quality properties.

Biography

Aline Zimmer is currently responsible for the development of mammalian cell culture media at Merck Millipore, based in Darmstadt, Germany. She holds an Engineering degree in biochemistry and biotechnology from the National Institute of Applied Sciences (INSA) in Lyon, France, a Master's degree in cellular physiopathology from the University of Lyon and a PhD degree in immunology from the University of Paris. She has worked 3 years in immunotherapy biomarker research at Stallergenes before joining Merck KGaA in 2011. From 2011 to 2012, she was responsible for the development of cellular assays to monitor upstream processes at Merck Millipore. From 2013 to 2014, she was heading the R&D Upstream Media Performance Laboratory responsible mainly for the development of new chemically modified amino acids and their

integration in fed-batch platforms. Since April 2014, she has been heading the mammalian cell culture media development activities at Merck Millipore.

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