Master of Science HES-SO in Life Sciences

DoE-based development of a production platform for multispecific antibodies

Marine Modoux

APPLIED BIOSCIENCES

HES-SO Valais

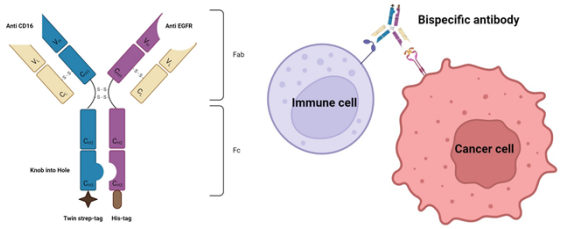
Advisor: Pr. Dr. Gerrit Hagens

DESCRIPTION

Monospecific monoclonal antibodies are widely used in cancer therapy because of their high specificity compared to other treatments such as chemotherapy. Antibodies offer different modes of action such as blocking molecules cancer cells need to grow, flagging cancer cells for destruction by the body's immune system, or delivering harmful substances (e.g., drugs, toxins, or radioactive substances) to cancer cells.

The interest for multispecific monoclonal antibodies, such as bispecific antibodies, is increasing because they offer advantages over monospecific antibodies. Indeed, their ability to bind to two different epitopes allows different action modes such as recruiting effector immune cells (e.g. T-lymphocyte) against a specific disease-related antigen, targeting two different ligands simultaneously, or aggregating simultaneously to two receptors to either inhibit or activate signaling pathways. These activities enable these therapies to be more effective than monospecific Abs/mAbs/antibodies, especially for the treatment of solid tumors.

This master work uses a bsAb that can recrute on specific immune cell and retargeting them to cancer cells



OBJECTIFS

The aim of this work was to optimize the production of this bispecific antibody in small-scale, large-scale shake flasks as well as evaluate different purification strategies to improve the yield of heterodimers.

In detail, the first objective was identifying parameters for bsAb titer optimization using Design of Experiment (DoE) method with focus on five different parameters (temperature shift, day of the temperature shift, feeding strategy, supplementation with FunctionMAX™, and agitation rate) for small shake flask CHO cultures. The second objective was the implementation of those selected parameters for cultures of higher volumes in shake flasks to see if the scale-up is applicable. The third and last objective was evaluating different purification strategies for the bsAb with the aim to increase the yield of heterodimers.

The DoE was executed using the MODDE software provided by Sartorius. It allowed the determination of process parameters that have an impact on the critical quality attributes of the cultures (titer, viable cell density) in small shake flasks (125 mL), and the determination of the optimal level of the important parameters for the obtention of the highest titer. The set up of the parameters was then executed in larger volume shake flasks (250, 500, and 1'000 mL).

Parameterers optimization **Scale-up**

RESULTS

Two screenings were done to determine impact of five process parameters on critical quality attributes of the culture of one CHO-S cell line. They were executed with powers of 96 and 97. The main response was the final titer [mg/L] of the cultivation, with the aim to increase this value.

The first screening focused of feeding strategy, temperature shift to lower temperature, and day of the temperature shift. It has been determined that day of temperature shift did not impact the titer, and feeding strategy and temperature should be at 2% and 37°C, respectively (Figure). The second screening focused on feeding strategy, FunctionMAX™ supplementation, and agitation rate. It determined that feeding strategy, FunctionMAX™ addition, and agitation rate should be at 2%, 0%, and 110 rpm, respectively,for the highest titer (400 mg/L).

The scale-up objective was realized in shake flasks of 250, 500, and 1'000mL with the strategy of keeping a constant mass transfer of oxygen (kLa). The curves obtained for the viable cell density, the viability, and the titer were the same for the three volumes as well as the 125-mL shake-flasks. The scale-up was thus applicable.

The yields of 84% and 54% were obtained by two different column for mAb capture with protein A.

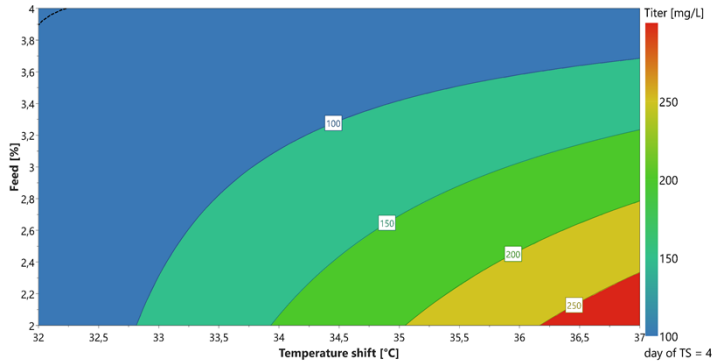


Figure 2: Contour plot providing final titer regarding the temperature and the feeding strategy for the CHO-S cell line.

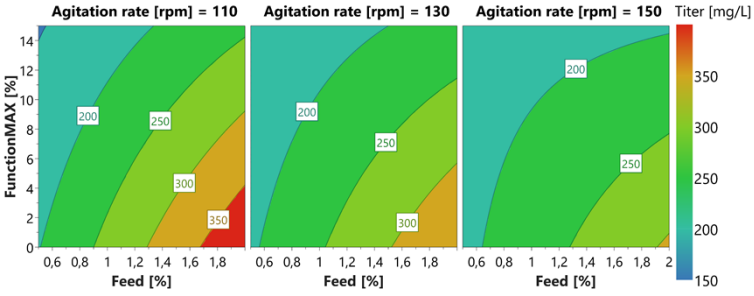


Figure 3: Contour plot providing final titer values regarding the feeding strategy, FunctionMAX addition, and agitation rate for the CHO-S cell line.

CONCLUSION

The DoE method allowed screening of five process parameters. This screening strategy brought optimal conditions for the CHO-S cell line cultivations with 2 % and 0.2 % of feed 1 and 2, respectively, environmental temperature of 37 °C with agitation rate of 110 rpm that provided a titer of 400 mg/L with a VCD of more than 25E6 cells/mL and less than 2 % of aggregates. The successful scale up showed that optimal conditions of cultivations determined in small shake flasks can be implemented in shake flasks of larger scale, by however considering a scale up strategy such as keeping the mass transfer of oxygen kLa constant. Finally, the efficiency for mAb capture of two different columns was evaluated and provided yield > 80% for the Fibro HiTrap™ MabSelect™ PrismA column, but other steps must be considered to purify the bsAb from all other impurities. Implementation of the optimal conditions for this bsAb producing cell line into larger scale of shake flasks and even bioreactor is the next step for the development of this production platform.