MYCENAX Article

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Process Optimization: The Key To Enhancing Yield And Quality In Bispecific Antibody Production

Bispecific antibodies (BsAbs) pose inherent challenges in expression, often leading to issues such as aggregation, fragmentation, and the generation of diverse charge variants due to their complex structures. Consequently, optimizing the upstream process for BsAb production is imperative. This optimization involves meticulous steps aimed at refining the production process, ultimately enhancing both the yield and quality of the final product.

Key Considerations in Upstream Process Development and BsAb Production

Below we outline the key considerations in upstream process development and BsAb production:

- **Cell clone selection:** Choosing appropriate cell clones for BsAb expression is crucial. Factors such as growth rate, specific productivity, and antibody product quality directly impact expression levels, stability, and the effectiveness of subsequent purification steps.
- **Culture medium:** The composition of the culture medium significantly influences cell growth, productivity, and antibody quality. Optimizing culture medium composition involves systematic media screening studies to obtain the best combination, evaluating the impact of components on cell growth, viability, and antibody production. Implementing fed-batch or perfusion strategies ensures continuous nutrient supply, maintaining optimal conditions throughout the culture.

- **Temperature:** Culture temperature plays a significant role in BsAb production, influencing cell growth, metabolism, and the quality of the final product. Optimization involves finding the temperature that maximizes BsAb expression levels without compromising product quality. Some clones may exhibit better BsAb production at specific temperature ranges, and careful temperature selection balances robust cell proliferation with optimal BsAb production.
- **pH:** Maintaining an optimal pH range is crucial for cell viability, metabolism, and proper BsAb folding. pH adjustment directly influences enzyme activity and antibody stability. Optimizing pH is essential for achieving high cell growth, maximizing BsAb yield, and ensuring product quality. Careful control and monitoring are crucial for maintaining consistent and high-quality BsAb production.

The success of BsAb production is intricately linked to the careful management of culture medium composition, pH levels, and temperature. These factors collectively influence cell growth, protein expression, and product quality. A well-optimized culture environment, considering the specific requirements of the chosen clones, is essential for achieving high and consistent BsAb yields with the desired quality attributes.

Optimizing Your Culture Environment

Ⅰ. Medium and Supplement Assessment

Exploring optimal conditions through screening, including pH adjustments, temperature optimization, and precise tuning of medium components, offers a promising avenue to enhance both BsAb production and product quality. Figure 1 illustrates the outcomes of a screening process focused on medium and supplements, revealing that varying nutritional combinations results in distinct product yield and quality profiles. Notably, productivity saw a substantial 3 to 5-fold increase depending on the specific medium and feed combination employed. However, it is crucial to consider the presence of high molecular weight (HMW) species. Despite the productivity gains observed with temperature shifts, there was an associated increase in HMW%, emphasizing the need for a balanced approach. Understanding the potential benefits of culture temperature adjustments can contribute to optimizing cell productivity in BsAb production.

Figure 1. Medium and Supplements Screening: illustrating the outcomes of a screening process focused on varying nutritional combinations, revealing distinct product yield and quality profiles.

Leveraging the capabilities of the ambr15 high-throughput system, a comprehensive screening was conducted to optimize multiple parameters, including pH levels and supplement ratios. This advanced instrument facilitated a rapid and systematic exploration of various conditions, enabling precise adjustments tailored to each clone's unique requirements. The efficiency and precision of the ambr15 played a pivotal role in enhancing the screening process, setting the stage for a more refined and targeted approach.

Following this systematic screening approach, Figure 2 showcases the integration of the identified medium and supplement combinations into two distinct clones. Accompanied by adjustments in supplement ratios, the outcomes revealed diverse nutritional requirements, resulting in varied quality profiles under both high and low supplement concentrations. The strategic use of the ambr15 system significantly expedited this screening process, demonstrating its instrumental role in tailoring conditions for optimal BsAb production.

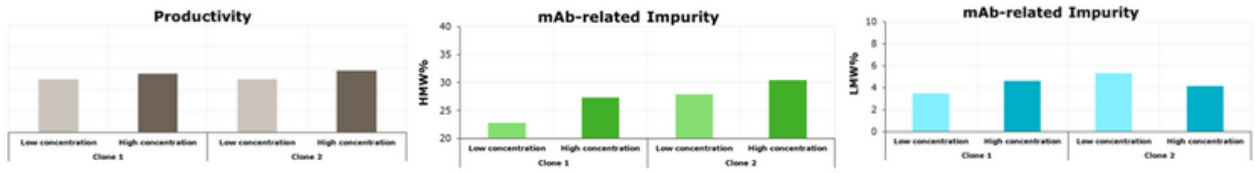

Figure 2: Feed Ratio Testing in Two Clones: the outcomes of feed ratio testing in two distinct clones, revealing diverse nutritional requirements and varied quality profiles under different supplement concentrations.

II . pH Modulation

Moving to another critical parameter, the influence of culture pH on both cell growth and BsAb production becomes evident. pH significantly shapes cellular processes such as metabolism, cell growth, and protein production. Furthermore, pH in the culture environment profoundly affects protein properties, making pH control crucial for mitigating adverse outcomes like protein aggregation. Figure 3 further emphasized the impact of pH modulation on BsAb production outcomes, revealing that both clones exhibited higher protein yields and lower HMW impurities under lower pH conditions. The strategic use of the ambr15 high-throughput system played a crucial role in achieving these findings efficiently. This advanced technology not only contributes to the optimization of BsAb production processes but also demonstrates its potential in accelerating the screening of numerous parameters, saving valuable time in the bioprocessing workflow.

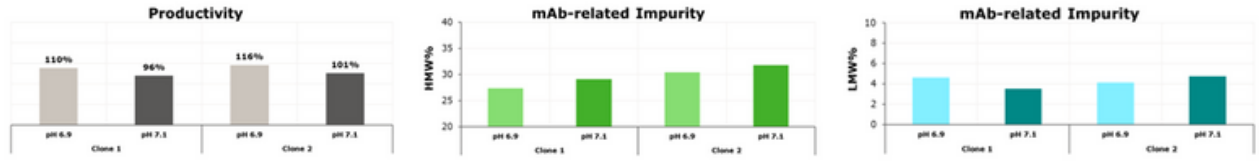

Figure 3: pH Modulation Impact on BsAb Production: highlighting the influence of pH adjustment on both cell growth and BsAb production, emphasizing higher yields and lower HMW impurities under lower pH conditions.

III. Bench-Top Bioreactor Confirmation

After the systematic screening of various parameters, including pH, supplement ratios, and culture temperature, using the efficient ambr15 high-throughput system, the identified optimal combinations were further confirmed and fine-tuned in a bench-top bioreactor. This transition to the bench-top bioreactor served as a crucial step to validate the findings from the screening phase and to establish a robust culture process for BsAb production.

With these optimized parameters in hand, the confirmation step in the bench-top bioreactor became paramount. Figure 4 highlights the outcomes of this confirmation step, emphasizing the significance of the cultivation period in influencing product quality. The results indicated that a prolonged cultivation period led to a higher HMW species ratio along with a higher yield. This finding underscores the importance of carefully considering cultivation duration in the bioprocess, with implications for both productivity and product quality.

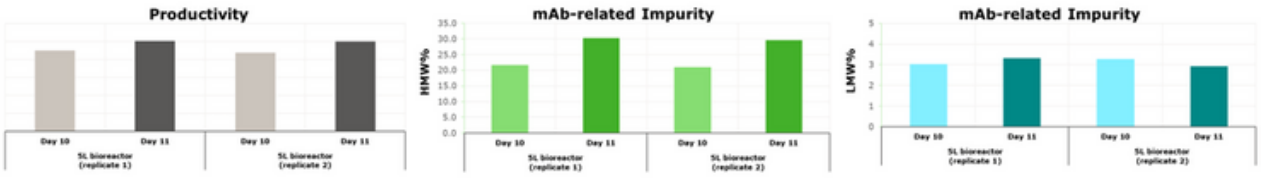

Figure 4: Bench-Top Bioreactor Confirmation: the significance of cultivation duration in influencing product quality, with a higher HMW species ratio observed with prolonged cultivation.

Build a Reliable Upsteam Process

In summary, our research strives to establish a reliable and efficient upstream development process for BsAb production, seamlessly integrating screening methodologies, advanced technology (ambr15), and bench-top bioreactor confirmation. This holistic approach not only enhances production efficiency but also lays a robust foundation for high-quality BsAb products. The insights gained from this integrated methodology provide valuable guidance for future research in bioprocessing, contributing to the advancement of upstream development.

About Mycenax

Mycenax is a dedicated CDMO specializing in process development and cGMP manufacturing of biologics and biosimilars. We provide integrated DNA-to-Drug Product services for mAbs, ADCs, plasmid DNA, cell therapies, vaccines, and more through advanced platforms and PIC/S GMP-compliant facilities. Our GMP site is approved by EMA, MHRA, PMDA, Health Canada and MFDS, and has successfully passed over 70 global client audits, ensuring the highest standards of quality and compliance.