Whitepaper: Advancements in microbial manufacturing of biopharmaceuticals

Advancements in Microbial Manufacturing of Biopharmaceuticals: A Historical Perspective and Future Outlook

Executive Summary

This whitepaper digs into the past, present, and future of microbial manufacturing in the biopharmaceutical industry. It begins with a historical perspective, highlighting the significant milestones in the field, from the production of insulin using recombinant DNA technology and how it participated to the advancement of microbial manufacturing processes.

The paper also sheds light on Boehringer Ingelheim's contributions to this field. It discusses the company's expertise in developing, manufacturing, and marketing products, which are produced in microbials.

A comparison between microbial and mammalian cell culture-based technologies is presented. It outlines the unique benefits and hurdles of each system. It underscores the flexibility of microbial systems to produce a broad variety of recombinant molecules.

The paper concludes with an outlook into the future of biopharmaceutical production. It highlights the potential of digital tools to enhance development and manufacturing processes.

A brief history

The use of microorganisms in making biopharmaceuticals started in the early 20th century. Back then, these organisms were used to make antibiotics. But the real game-changer arrived in the 1970s with the introduction of recombinant DNA technology.

One of the significant achievements in microbial manufacturing of biopharmaceuticals was the production of insulin using recombinant DNA technology in the late 1970s. This was the first instance of a human protein being manufactured using a microorganism. It paved the way to the production of other recombinant proteins.

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Fast forward to the 1980s and 1990s, advancements in genetic engineering and fermentation technology led to the development of more sophisticated microbial manufacturing processes. Microorganisms like *E. coli* and yeast systems (mainly *S. cerevisiae* and *P. pastoris*) became the go-to choices for producing a wide variety of biopharmaceuticals. These included growth hormones, clotting factors, and antibody fragments.

Boehringer Ingelheim takes pride in being a pioneer in the field. The company started with development, manufacturing, and marketing of recombinant interferons in the early 80s. Since then, 20 microbially produced medicines were brought to the market. This portfolio includes own products as well as a broad range of different modalities manufactured for third-party customers in the contract manufacturing business.

Advantages and disadvantages of the different Host systems

Today, biopharmaceuticals are primarily produced in two types of systems: microbial systems (*E. coli* and yeasts) and mammalian systems (most commonly Chinese Hamster Ovary cells, CHO). Mammalian systems are used for making large molecules and molecules that require human-like post-translational modifications such as glycosylation. Monoclonal antibodies, which are currently the most prominent class of molecules, are typically produced in CHO cells. On the other hand, microbial systems are utilized to produce a wide variety of molecules. These can vary greatly in size and function, from peptides to plasmid DNA (pDNA). However, it is important to note that products made in microbials are typically not glycosylated.

Even though microbial systems cannot perform human-like post-translational modifications, they have significant advantages when it comes to the production of non-glycosylated modalities. Cultivating microbes is typically much faster than mammalian cell cultures. A typical microbial cultivation process takes just a few days, while CHO cultivation can take several weeks. Furthermore, microbes are very robust and can be cultivated to very high cell densities with comparatively simple and cost-efficient cultivation media.

The development of mammalian manufacturing processes has been focused on establishing efficient platform technologies for monoclonal antibodies. These processes rely on fed-batch fermentation processes, followed by a common affinity capture step. This step helps to purify the protein of interest from the cell culture supernatant. Further purification steps include filtration and chromatography. Yet, while a wide variety of molecule formats can be produced via fast cultivation in microbial systems, these present their own challenges. They require tailor-made processes, which can be addressed with platform-like technology modules and a versatile toolbox to serve individual needs.

Microbial toolbox for efficient and tailored manufacturing processes

Microbial systems are used to produce a variety of proteins, with different methods for expression and purification. In the bacterium *Escherichia coli,* the product can either accumulate in the cytosol in a soluble form or as insoluble inclusion bodies (IBs). Alternatively, products can be targeted to the periplasmic space to allow for the formation of disulfide bridges. Although IBs can be produced in large quantities, they require additional process steps to unfold and refold the protein of interest into its native state. These steps can be time consuming, potentially limit throughput, and may lead to product losses. The manufacturing of correctly folded, soluble products can be challenging due to very low titers or even be impossible. In all scenarios, the molecule of interest needs to be released from the cells by cell disintegration, typically achieved via high pressure homogenization. Yeast systems like *Komagataella*

phaffii (formerly known as *Pichia pastoris*) allow for the secretion of the soluble protein of interest into the media. Independent of the expression strategy, the need for a custom purification process increases the complexity in process development and manufacturing in multi-product facilities.

Technologies that simplify processes by introducing platform-like steps are beneficial for streamlining development and manufacturing, and for increasing productivity. Boehringer Ingelheim has developed one such technology, the CASPON™ fusion protein approach, for non-platform molecules (see Figure 1). The CASPON™ system includes a peptide fused to the N-terminus of the protein of interest and a highly specific protease to remove the tag after purification, leaving the authentic N-terminus of the target protein [1]. The tag serves multiple purposes: it increases product yield (up to ~10 fold depending on the target molecule), enhances product solubility (preventing IB formation), provides a recognition sequence for the protease, and includes a histidine stretch for a platform affinity purification. The protease is specifically designed to cleave at the recognition sequence and is flexible regarding the N-terminal amino acid of the protein of interest, allowing for broad molecule application [WO21028590], [2].

The CASPON™ system and similar technologies simplify purification and boost productivity. Additionally, there are numerous genetic tools available to enhance microbial system productivity. Boehringer has developed a comprehensive toolbox including strains, plasmids, promotors, leader sequences, and helper factors (see Figure 2). This toolbox has been efficiently used to improve soluble expression in *E. coli* by more than 25-fold (see Figure 3).

The full potential of this versatile toolbox can only be realized with a predictive screening system. However, typical screening systems in deep well plates or shake flask cultures often do not accurately predict outcomes for large scale high cell density fermentations, especially for *E. coli.* To address this, Boehringer Ingelheim has developed a miniaturized fermentation system in the milliliter scale that allows for predictive screening of different constructs in an automated, parallel manner [3]. This complemented by miniaturized, parallelized, and automated screening systems for purification and analytics, enables high throughput process development.

A predictive system is essential for selecting the best production strains and for development of robust processes and scaling them up. Boehringer Ingelheim uses tools like computational fluidic dynamics (CFD) to characterize vessels in different scales and adapt small scale equipment to best mimic the large-scale production environment [4].

Digital tools have a significant potential to further improve development and manufacturing of biopharmaceuticals. CFD simulations and process models can predict process performance across scales and help optimize process conditions [5]. Process models can also reduce lab work during process development and characterization studies [6]. Furthermore, *in-silico* process models provide a holistic approach to process development, considering interactions between several united operations. Classical process development focused on the optimization of each unit operation, which may not yield the best overall process. Too fully leverage statistical, mechanistic, and hybrid modeling techniques, Boehringer Ingelheim has developed a modelling platform [7].

Outlook & Conclusion

Microbial Manufacturing Technologies offer a powerful means to produce a diverse array of products. Despite the use of microbial systems in biopharmaceutical production since the late 70s, challenges persist due to the wide variety of modalities and increasing demands for cost efficiency and speed to

market. In our view, an efficient microbial process is based on a comprehensive and well-understood toolbox of manufacturing technologies, coupled with a predictive screening approach. Platform-like technologies for non-platform molecules, such as the CASPON™ system can significantly enhance the use of microbial hosts. Digital tools for characterization and prediction can minimize the needs for wet-lab experiments, identify optimal points throughout the entire process chain, and contribute significantly to the development of robust, well-understood processes. They also facilitate seamless transfer across scales from milliliters to cubic meter.

The Integration of such digital tools with appropriate in-line and at-line analytical technologies (PAT) will define the future of biopharmaceutical process development and execution. This could potentially reduce the experimental need for process development, lead to fully automated 'light-out' facilities, and enable real-time release concepts.

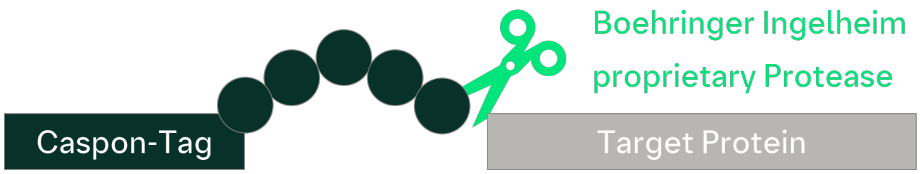

Figure 1: Schematic depiction of the CASPON™ Technology

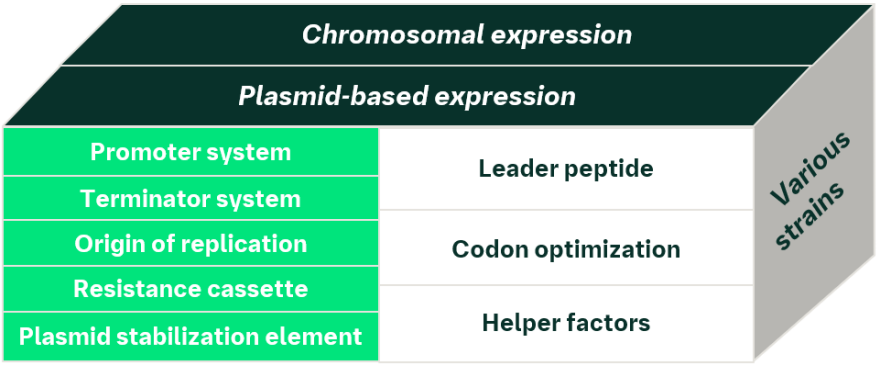

Figure 2: Molecular Toolbox

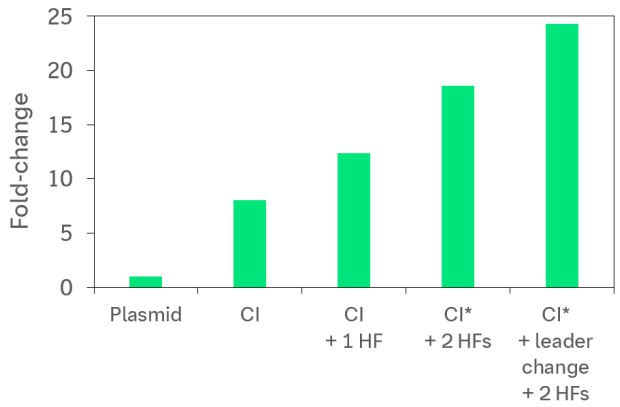

Figure 3: Fold-change of a scFv titer using selected molecular tools in fermentation (*further process adaptation)

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