

Metabolic control of CHO^{BC}[®] for production of biologics

CDMO Process optimisation for biologics is obligatory for efficient processing and reduction of Cost of Goods (COGs). Especially in the development of biosimilar antibodies, low COGs to manufacture the antibody provides a competitive advantage compared to competing biosimilar developers. The major advantage of our own CHO^{BC}[®] platform is that all available expertise and newly obtained knowledge can be directly leveraged for process optimization.

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During the last decade, process development for biologics has evolved into a highly professional expertise resulting in high titer processes, along with decreasing COGs. However, only limited amounts of the available knowledge from 60 years of literature is currently being used to understand the intracellular metabolism of the used host cell lines. Although spent medium-analysis provides information on extracellular metabolite concentrations, all the metabolic action relevant for the quality of the product occurs intracellularly at completely different metabolite concentrations. Therefore, metabolic understanding and, more importantly, metabolic control of our CHO^{BC}[®] platform has been developed. This facilitates easy transfer to the develop-

ment laboratories in Gdansk at Polpharma Biologics and allows us to produce clinical trial material at our GMP suite. This direct interfacility collaboration and process understanding is the foundation for development of GMP processes to be transferred to the large manufacturing facility, which is currently being built in Duchnice, located near Warsaw, Poland. The newly built facility of 30,000 m² is based on disposable fermentation and equipped with state-of-the-art hardware.

SPOT[™] for optimal productivity

In current practice, the method used most often by the upstream process (USP) development teams to achieve high produc-

tivity is to increase the viable cell density (VCD) in their upstream processes. Although high VCD results in higher product titers, this strategy also creates challenges for the downstream processing (DSP) teams, since clarification of more cells is more difficult and requires for high filter surfaces. In addition, higher VCD also results in higher host-cell related impurities which must be removed by DSP. Therefore, we developed USP technologies to increase volumetric productivity, without a major increase in VCD, by increasing the cell specific productivity of production cell lines. For this reason, the proprietary SPOT[™] technology was developed, which aims to increase specific productivity per cell rather than the current USP optimization, resulting in higher VCD. This technology has been used in various programs and increases specific productivity (up to 200 pg/cell/day) and ultimately therefore also increases volumetric productivity up to 400% in various programs (up to 8 g/L; Figure 1). An additional technology, which was developed to increase specific productivity, is based on increasing the cellular volume rather than increasing the VCD. Using this strategy, total biomass increases in the absence of major VCD increases. Examples to be used in the process to induce the increase in cell volume are amino acids like citrulline and a sugar like sucrose. The use of citrulline decreased the VCD in the USP process of our ustekinumab program for 60% at comparable productivity as in the

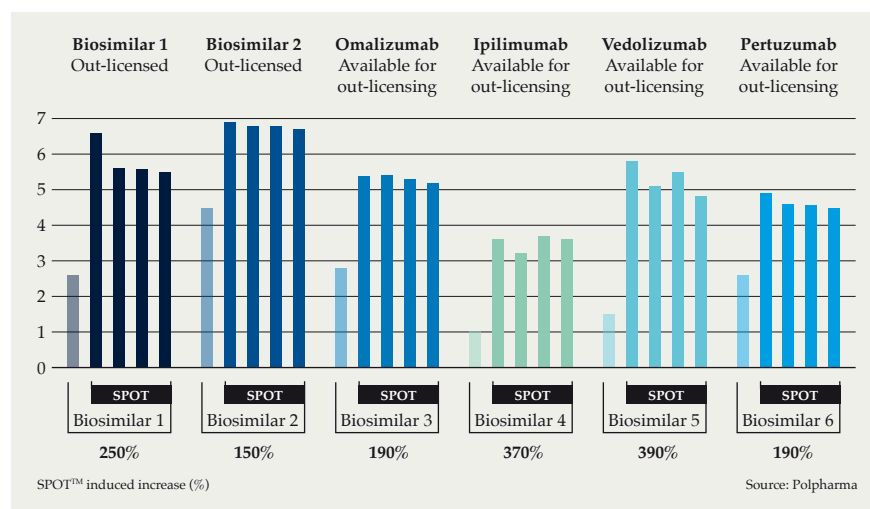


Figure 1: SPOT[™] induced productivity increase. Compare first transparent bar of every color with the others of that color.

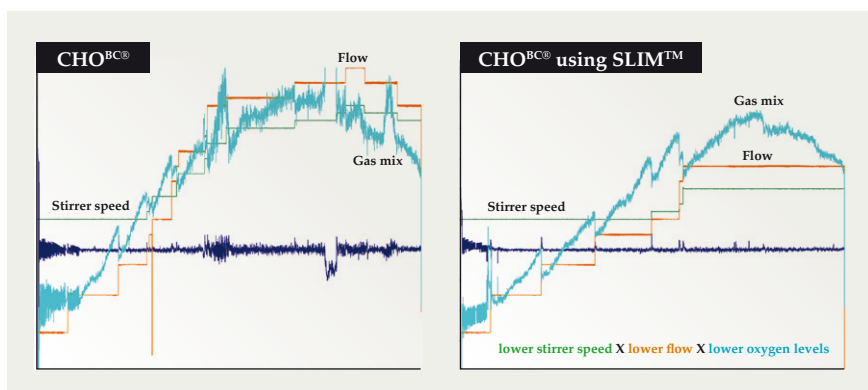


Figure 2: SLIMTM technology improves process efficiency. Compare the flow rates (orange lines), the stirrer speed (green line), and the oxygen level in the gas mix (light blue line) of CHO^{BC} (left) to CHO^{BC} using SLIMTM (right).

absence of citrulline. Reductions of host cell protein content in the presence of citrulline were 40%, while the filterability for clarification was increased by 40%/gram of product. The latter has huge consequences for the GMP footprint of the clarification filters and, furthermore, reduces the dead volume of the clarification filters, resulting in less product loss, and consequently lower COGs.

SLIMTM for efficient bioprocessing

One of the major drawbacks of current bioprocessing is that all high-producing processes demand high sparging and agitation rates and moreover consume high amounts of feed and oxygen. Besides pushing the current bioreactor systems to the limit, the induction of shear stress and product oxidation in current bioprocessing raises questions about the sustainability of current bioprocessing. Moreover, since the feed of the process is an expensive part of total production costs, the high consumption increases COGs. To find a solution for these process challenges, we leveraged our intracellular metabolic understanding of our CHO^{BC} platform to perform metabolic engineering (in the absence of any genetic manipulation). The outcome of these studies resulted in our SLIMTM technology, which enables our CHO^{BC} host cell line to become much more energy efficient and therefore more sus-

tainable in the future. The SLIMTM technology facilitates better transfectability and supports single cell growth, enabling better outgrowth of cell lines. Confirmative to our metabolic engineering design, in bioreactors the SLIMTM technology reduces the need for high agitation and sparging rates and reduces the oxygen and feed consumption (Figure 2). This results in better product quality at lower prices and far away from bioreactor hardware limits.

CHO^{BC} CQA modulation toolbox

While using the SPOTTM technology, specific productivity is increased and the SLIMTM technology improved the efficiency of the process – the upstream scientists leverage the platform knowledge and metabolic understanding to design and develop a process modulation toolbox to specifically modify CQAs on the biosimilars that are not similar to the originator profile. Upstream process technology was developed to specifically increase or decrease specific glycan structures on antibodies. Methods were developed to specifically modify terminal galactosylation, core-fucosylation, and mannosylation on the biosimilar. In addition, methods were developed to influence the charge profiles of the biosimilar, including decreasing acidic variants and increasing/decreasing basic variants. Interestingly, the above described citrul-

line addition to the process not only results in cell swelling and reduction of VCD, but also in a reduction of acid variants and an increase in basic variants. These modulatory strategies, which do not limit productivity, deliver high producing biosimilar antibody processes, enabling us to ultimately produce those biosimilar drugs at low COGs. The developed modulation toolbox can also be used beyond biosimilars for novel biological entities. Since high terminal galactosylation on antibodies can increase their CDC effector function and low core-fucosylation can improve the ADCC effector function, the modulation toolbox can obviously also be used to tweak the anticipated biological effectiveness of a novel product. For a mechanism of action of a therapeutic antibody that needs effector functions, the USP process can be modulated to high terminal galactosylation or low core-fucosylation, while for a novel antagonist antibody the reverse can be designed in the USP process. These simple USP adjustments to the production process of a therapeutic antibody can eliminate the need for coupling of toxic compounds, as been done in ADC, to increase the cell-killing potential of the targeting antibody.

The above described SPOTTM and SLIMTM technologies and the process modulation toolbox clearly show the value of detailed knowledge of the intracellular mechanism by which the therapeutic protein of interest is being produced and its quality is modified. Conclusively, the extensive and ever-increasing knowledge about our CHO^{BC} platform is and will be directly leveraged in our USP processes to generate high amounts at an optimal quality of therapeutic proteins. The Polpharma Biologics groups will develop a broad portfolio of high producing biosimilar processes, which will be used to strengthen our internal biosimilar pipeline, but are also available for out-licensing purposes. The extensive platform knowledge and the flexible integration of the group members is also available for customers who wish to profit from our in-depth manufacturing expertise and flexible capacities. ■