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Assessment Of The Impact Of The Milling Type And Production Site For Cell Culture Feed Production

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Context And Introduction

The current process to produce a protein of interest at Ichnos starts with the expansion of the clonally derived manufacturing cell population of mammalian cells (Chinese hamster cells) from a cryovial followed by the fed-batch step, the major step of the upstream process. During the fed-batch, usually biomass is accumulated until a switch in the type of metabolism is induced in order to make the cells produce as much of the protein of as possible. Consistent quality of cell culture media and feeds is highly important to reach good performances in the manufacturing processes. Any change in these critical raw materials can have a tremendous effect on product quantity and quality.

Some commercial media and feed suppliers have multiple production sites using different manufacturing technologies. In order to claim for comparability of their products the Certificates of Analysis (CoA) are harmonized across different sites. In addition to the CoA, additional tests like cell culture performance tests with internal cell lines may be performed. Following a change in the powder milling process at the supplier site, Ichnos performed additional tests on feeds derived from different production sites using different cell lines. This approach would allow to anticipate and derisk any potential effect specific to the Ichnos process, that may not have been identified by the supplier during its study.

Description Of The Media And Feed Production Process

To better understand which steps of the media and feed powder manufacturing process might have detrimental effects on performance, this section will describe the main steps of the manufacturing process.

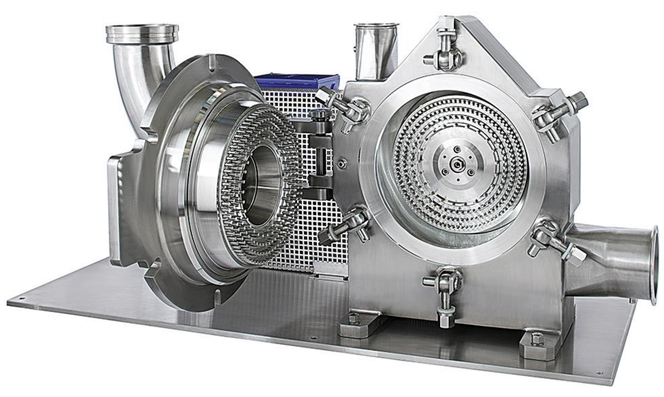

Figure 1: One of the most common mills is the Hosokawa mill. Its main characteristic is to have pins to mill that allow a homogenous granulometry of the final product

For a worldwide distribution of the final products, all the raw materials need to be animal component free and comply with the US and European pharmacopeia. First all the ingredients are weighed. Trace elements must be added using liquid sprayed on larger powder particles to ensure a good distribution. Then everything is mixed a first time to distribute the components. This is followed by a milling (Figure 1) step that will allow to reduce all material to a smaller size which would be easier to homogenize and dissolve. The final step is another round of mixing that leads to a uniform repartition of the different components.

The powder supplier provides a Certificate of analysis that includes different physical and chemical analyses to ensure consistent quality of its product. On the biological side, a toxicity test is routinely performed.

Material And Methods

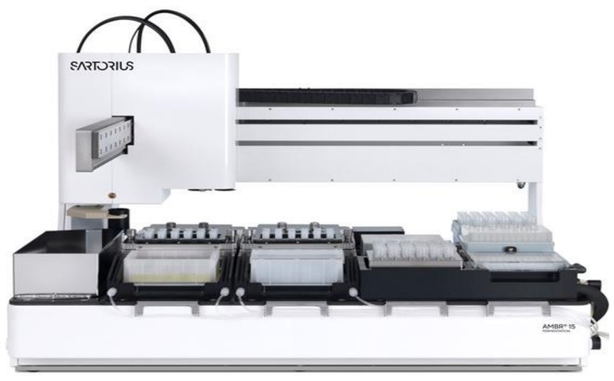

Figure 2: Ambr 15 ® with 24 microbioreactors

The supplier sells powder and liquid form of their product. In this derisking study, however, only powder products were available. The supplier protocol was followed for the reconstitution of the feed.

This experiment was run with 3 different projects and cell lines: Project A, Project B and Project C. Two different formats of molecules were selected.

The same reference of a raw material produced at two different sites was tested and is referred to as “product A” and “product B”. These were coming from two different milling types to validate all the different manufacturing processes available with this media and feed supplier. The current cell culture platform process was used for all the projects. Each combination was run in triplicate.

The bioreactor used for this experiment was an Ambr 15® (Figure 2). The Ambr 15® is composed of a workstation linked with a liquid handler controlled by a laptop. This enables automated feeding and sampling, saving time of handling from the operator. Bioreactors were filled with only 15mL. This allowed to drastically reduce the volumes required for media and feeds that are important contributors to the cost of a run. The different cell parameters and metabolites were first compared graphically, followed by a statistical comparison of the process performance using the product titer as a main indicator.

Comparison Of The Cell Culture Parameters And Metabolites For Project A

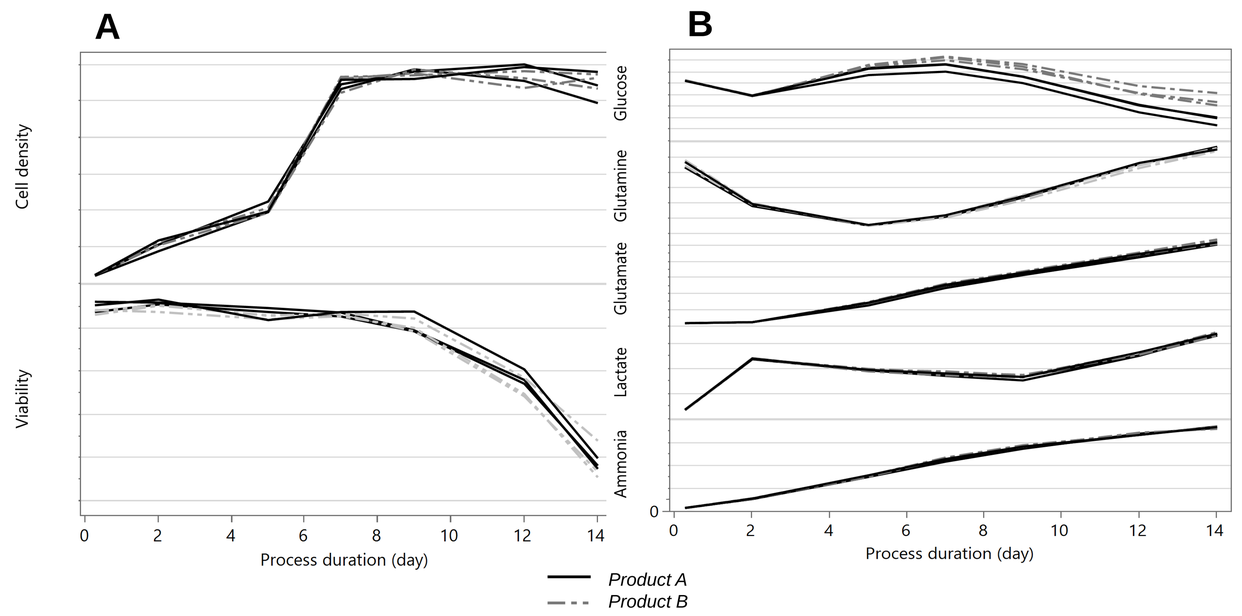

Figure 3: (A) Cell culture parameters for Project A : cell density and viability. (B) Metabolites for Project A : glucose, glutamine, glutamate, lactate and ammonia

Focusing on the cell culture parameters, growth is highly similar between the two products during the whole run. Peak cell intensities are equivalent, and the cell maintenance is identical between the different bioreactors. There is no early drop of viability, and the process was stopped at the typical platform harvest timing.
The trends of the different metabolites are very close throughout the whole run. The glucose trends are slightly different at the end of the run.

Statistical Comparison Of The Final Titer For Project A

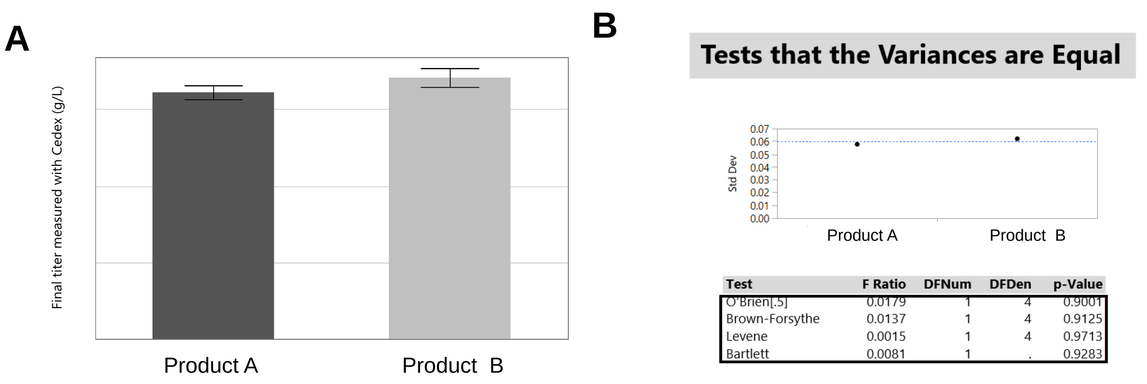

Figure 4: (A) Titer plot measured by Cedex equipment for Project A. (B) Results of Test of the equality of the variance for Project A (C) Results of the pooled t-Test for Project A

Graphically, it appears that there is only a negligible difference between the two sets of bioreactors / raw materials (A). A statistical comparison allows to determine a more reliable conclusion. The analysis is composed of two steps: a verification of the equality of the group’s variance, then a pooled t-Test on the two groups. Different statistical tests were performed on the variance of the group. As all tests have a p-value above 0.05, it can be concluded that the group variance is equal (B). This allows to perform a pooled t-test. The resulting p-value of the t-test is above 0.05 (C). This result allows to conclude that there is no statistical difference in titer between the two groups of bioreactor runs with the two products.

Final product quality was also assessed and found to be comparable between Product A and Product B for Project A.

This analysis was also performed on the two other projects (Project B and Project C) and comparable results were obtained.

Conclusions

- There were no overall differences between Product A and Product B on Project A performance and product quality.
- No statistically significant differences were observed in terms of titer between the two products.
- Similar results were also confirmed with the Project B and Project C.
- Product A and Product B from two different sites can be used interchangeably.

This study will allow business continuity in the manufacturing process when a switch from a supplier manufacturing site to another site is required.