CASE STUDY: SCALE-UP CHALLENGES OF E. COLI PROTEIN PRODUCTION PROCESS

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SITUATION

Scale-up of biomanufacturing systems continues to be a challenge. In this situation, we sold the process and the product to a licensee, and they decided to scale it up right away.

We had been operating it at a relatively small scale in the lab and got it working well but when it was scaled up, the product came in at just 50% of the normal potency so it was clear there was something wrong with the product.

ABOUT

Parrish has over 30 years of experience in bioprocessing, during which time he has helped build development, manufacturing and CMC teams who developed scalable bioprocesses, built biomanufacturing facilities, and achieved commercial licensure.

In this case study, Parrish shares his experiences of investigating scale-up setbacks and unexpected product integrity issues.

CHALLENGE

The challenge was to figure out what was causing this drop in potency. There were four possible areas to investigate, and we embarked on studies to determine the reason and avoid a potential patient risk due to changed proteins:

1. Media
2. Feeds - feed batch
3. Bioreactor operations conditions
4. Age of cell line

We first looked at the limitations of the process development work we had done, and it was apparent we were working with media that was variable in quality and amino acid composition. Up until this point, we had done small-scale engineering studies in the bioreactors but not any simulation work to simulate large-scale. We were not running the process for very long, so we were not asking the cell line to go through many doublings in the bioreactor. These were the things we systematically did studies around.

SOLUTION

We designed scaled-down studies that we could do in the lab that would simulate what the cell was going through in the very large-scale bioreactor. That meant looking at the duration of the run, how many doublings the cell line was going through and the consumption of the media components in that process. We also simulated the engineering performance of the large scale bioreactor, whereby we modelled the engineering aspects of the bioreactor – the power input, the mass transfer rates and the mixing times – and duplicated those in the laboratory bioreactors. From this, we were able to eliminate engineering scale-up parameters as a root cause.

When the customer went into a large-scale bioreactor, they grew the cells for many more generations than we did in the lab, which brought into question whether the gene coding for the product was stable. We analysed cell samples taken from the bioreactor and examined the gene coding for the product, the conclusion was that the gene itself did not change over the course of the run.

The next question was whether there were any changes in the mRNA made from the gene coding DNA. When we did Northern blot studies to look at the mRNA quality and the sequence, we found there were changes in the mRNA that were not expected. In fact, when we sequenced the protein that was 50% less active, it turned out the sequence had changed, so clearly something was going on regarding translation. That eliminated the question about the DNA being corrupted over long doublings, but it raised the question of why the cell was inserting incorrect amino acids into the sequence of the product.

The final step was to go back and look at the media because by growing in the bioreactor longer in the large-scale system, the cells were consuming more of the media components to both grow E. coli cells and make the product. We did a mass balance calculation to understand how many cells were needed, how much product we wanted to make, and the amount of amino acid required to do both. What we found was that the media used in the large-scale bioreactor did not contain sufficient amino acids to produce both E. coli cell protein and the product.

In conclusion: there was a limitation in the amino acid content in the media used in the large-scale bioreactor.

Outcomes

We used what was a new technology at the time – high throughput screening – and set up a lab DoE experiment to screen media formulations and vary the amino acid compositions, especially for those amino acids identified to be in short supply in the large reactor. What that revealed was four or five different amino acids were running out and when cells are faced with the starvation of amino acids, they can insert different amino acids into the sequence because there's a wobble in the tRNA to allow for that. So, the cells were doing what they would normally do in this situation – scavenging different amino acids to produce the protein. It was this altered amino sequence that led to a reduction in potency of 50%.

We came up with a new media formulation, which was a fed-batch formulation that would augment the media as the cells were growing over an extended period and we transferred that knowledge to the customer and implemented it in their large-scale system. The result was positive, the outcome was that the cells produced the right product with the right potency.

The downstream purification was also modified because when the protein had the wrong amino acid sequence, it had a different isoelectric point and behaved differently in the purification chromatography steps than it did in the lab. When we corrected this and started producing the correct protein, the purification in the downstream area went back to normal and we were able to recover good yields of the right product.

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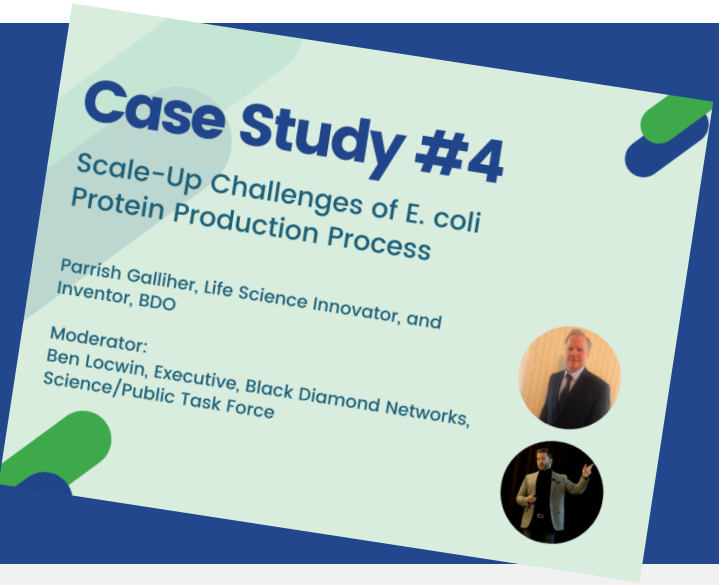
LESSONS

When you're going to scale up, do your homework about what will scale up in an expected fashion and variables there could be that lead to an unexpected result.



This case study was presented at a recent virtual event 'Technology's Evolution and Impact on Manufacturing', which included six in depth case studies and networking sessions.

Details of future events can be found here.

You can watch Parrish's case study in full and on demand here

ABOUT EVALUATING BIOPHARMA

Evaluating Biopharma is a convener of knowledge, data, and industry leaders within the biopharma and bioprocessing industries. Built upon the foundation of BioPlan Associates decades of data collection and analysis,

Evaluating Biopharma brings together top industry experts, innovators, decision-makers, and leading providers so that together they can share, evaluate and discuss critical topics that will help biopharma and bioprocessing leaders advance life sciences.

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