A Popular Summary on:

Development of an Online Monitoring and Sampling Scheme for Recombinant Protein Production

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Strive for innovation. The development of online sampling and monitoring schemes for fermentation implementation resulted in fast protein recovery and an applied translational tool for dilutions. The goal, to further the recommendation of innovation for the pharmaceutical industry.

To travel deeper into the underlying interest of developing process monitoring and sampling techniques is not limited to a narrow scientific community. The strive for industrial innovation in the pharmaceutical industry, including but not limited to sampling, is encouraged by the Food and Drug Administration's (FDA) directive, the Process Analytical Technology (PAT).

Time is money, and the faster the information retrieved the quicker the response time, the greater chance of being proactive. Essentially, the sample must represent the conditions inside the bioreactor. That is one of the criteriums within Good Manufacturing Practice (GMP) by the European Commission. Therefore, in correlation, it should be attractive to know sooner rather than later the status of the reactor content.

To exemplify, in a fermentation of *Escherichia coli*, from the point of sampling from the reactor, it takes well over an hour to merely recover the protein of interest inside the organism. What if recovering the protein could be performed automatically within 19 minutes, using microliters instead of millilitres of samples and chemicals? The reproducibility, reduced time delay between sampling moment and retrieval of information from the analysis, possible feed-back control or early termination of a fermentation. Essentially, time saved is money saved.

To develop such an online automatic sampling scheme starts with an initiation scheme. The robustness of already developed programs by CapSenze, permits the implementation of an optical density monitoring step. The exiting part of implementing such a step is the online and real-time following of organism growth. Following this step, the user can add a sampling step where the organism is sampled, and the protein of interest purified. Further inspiration for the adventurer can be found in the created users guide for scheme creation.

The robustness of such sampling technology was strengthened by studying different features of the technology. Binding of the protein was successful at 30 mM imidazole, a chemical that hinders unwanted interactions during purification. Chemical lysis, even though minutes and microliters were used, indicated clear trends that increased time and ratio (to cells) improved the degree of degradation. To clarify, of the 0, 30, 60, 120 and 240 seconds of reaction in combination with an increasing ratio of 1:1 to 1:2 (cells to lysis reagent), the combination of increased time and ratio improved the results.

Following the progress as the cells grew, the monitoring scheme measured the optical density. To accurately follow the growth, the cells first had to diluted by the scheme through decreasing the injected volume. The creation of a dilution translation tool presented the advantage of recalculating the measurements to represent *Escherichia coli* fermentations from the set-up.

The diversity of the different studies and the rapid protein retrieval from the online monitoring and sampling scheme speak to the robustness of the VersAFlo. Saved time gives room for the decision-making process and the future implementation of a sensitive analytical tool, closes the circle for the developed scheme. Ultimately the goal is to strive for innovation.