Downstream Sampling Process Optimization

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Abstract *— The Downstream Sampling Process in the Biopharmaceutical industry is a timely and product consuming step. This process can be described as a highly labor intense due to all the required steps to collect a sample. In order to optimize the sampling process is important to maintain the product sample attributes. For this case these attributes were; pH conductivity, endotoxin and bioburden. The main objective of this project was the optimization of the product sampling process based on samples reduction, sterilization, and reducing the risk of product contamination by applying a six sigma methodology. PR Biotechnology Solutions Company challenged the capability of two in-process sampling devices (MN and GSD). Finally, experiments results, showed that both sampling devices MN and GSD are capable to collect the in process samples from process tank without affecting the established process parameters or compromising the sterility of the sample.*

Key Terms – *Downstream, Optimization, Product, Sampling.*

INTRODUCTION

Biologics parenteral process is divided in Upstream (Inoculum & Fermentation) and downstream (Capture & Purification) operations. Through the downstream and upstream process, the product is being monitored in different steps to determine the efficiency of the process step, product quality, and to verify that product integrity is maintained. The actual downstream sampling process is performed using a dedicated valve which is installed in each of the process tanks. During the sampling process, which is a labor intense activity, product is purged, making the sampling process a timely and product consuming step. The main objective of this project was to apply a six sigma methodology for the optimization of the product sampling process based on samples reduction, sterilization, and reducing the risk of product contamination.

Project Description

This project will be conducted at a PR Biotechnology Solutions Company. PR Biotechnology Solutions Company will like to challenge the capability of two in-process sampling devices (MN and GSD) without affecting the sterility, quality and efficiency of the process tanks sampling process. The results will be compared with the ones obtained from the actual sampling method (Dedicated tank valve).

Project Objectives

The objective of this project is to generate enough data after the DMAIC implementation with the intention to improve the sampling process, while the operation cost and cycle time is optimized.

Project Contributions

The project will contribute on the PR Biotechnology Solutions Company, product X10 process optimization. This alternative technique represents a process improvement of the sampling method it will reduce sample process time consumed and product loss reduction, maintaining product quality.

Literature Review

In order to understand this biotech downstream sampling optimization a general knowledge in Biotechnology, Product X10, downstream, Six Sigma, DMAIC, and sampling method must be discussed.

Biopharmaceutical Process

The modern pharmaceutical industry is barely 100 years old. Among the most recent product types developed are the biopharmaceuticals; therapeutic substances produced by modern biotechnological techniques. Thus far, in excess of 50 such substances have gained regulatory approval for medical use. All are proteins produced by recombinant DNA technology or (in the case of monoclonal antibodies) by hybridoma technology.

Biopharmaceuticals approved to date include blood factors, anticoagulants and thrombolytic agents, therapeutic enzymes, hormones and haemopoietic growth factors. Also approved are a number of interferons and an interleukin. Recombinant vaccines and several monoclonal antibody based products are also now on the market. In addition to these, in excess of 350 potential biopharmaceutical products are currently under evaluation in clinical trials. Prominent among these is a new sub-class of biopharmaceutical - nucleic acid. Nucleic acid based products find application in the emerging therapeutic techniques of gene therapy and anti-sense technology. These techniques will likely provide medical practitioners with an additional powerful tool with which to treat conditions such as genetic diseases, cancer and infectious diseases.

The biopharmaceutical sector will continue to grow strongly for the foreseeable future. Its current global market value of $7-$8 billion is likely to triple within the next 5-6 years. This sector, born less than 20 years ago, is quickly reaching maturity.

By the late 1970s, hundreds of startup biotechnological companies had been formed to develop such products. Most such ventures were founded in the USA, mainly by academics and technical experts in the biotech world. These companies were largely financed. When they boasted significant technical expertise, most of these companies lacked practical experience in the drug development process. In the earlier years, most of the established large pharmaceutical companies failed to appreciate the potential of biotechnology as a means to produce drugs and, consequently, were slow to invest in this technology. As its medical potential became apparent, many of these companies did diversify into this area of biotech. Efforts in house, most either acquired small established biopharmaceutical firms, or entered strategic alliances with them. An example of the latter was the alliance formed between Genentech and Eli Lilly with regard to the development and marketing of recombinant human insulin.

The vast majority of biopharmaceutical products currently on the market are produced by recombinant DNA technology in either E. coli or Chinese Hamster ovary (CHO) cell lines.

Most monoclonal antibody based products are predictably still produced by hybridoma technology, although the technical methodology now exists to facilitate production of antigen-binding antibody fragments by recombinant means.

E. coli represents a popular recombinant expression system for a number of reasons. In addition to its ease of culture and rapid growth rates, E. coli has long served as the model system of the prokaryotic geneticist. Its genetic characteristics are thus exceedingly well-characterized and reliable standard protocols for its genetic manipulation have been developed. Appropriate fermentation technology is well established, and high expression levels of recombinant proteins are generally attained. E. coli, however, does display some disadvantages as a recombinant production system.

Recombinant proteins generally accumulate intracellular, complicating downstream processing and (often more critically) E. coli lacks the ability to glycosylate proteins (or carry out any other post-translational modifications).

Many proteins of therapeutic interest are naturally glycosylated and lack of the carbohydrate component can, potentially affect its biological activity, solubility, or in vivo half-life.

Recombinant proteins may be expressed in a number of other microbial systems which do contain the enzymatic activities to facilitate

posttranslational processing. Various proteins have been expressed, both in yeast (particularly Saccharomyces cerevisiae) and fungi (especially various Aspergilli). Microorganisms are capable of glycosylating recombinant therapeutic proteins. The pattern of glycosylation usually differs to that associated with such proteins when expressed naturally in the human body. Microbial expression systems exhibit a number of characteristic advantages and disadvantages in terms of recombinant protein production. However, few recombinant biopharmaceuticals developed are produced in either yeast or fungal systems. Two approved biopharmaceuticals are produced in Saccharomyces cerevisiae: Refludan (recombinant hrudin, an anticoagulant marketed by Behringwerke AG) and recombinant hepatitis B surface antigen incorporated into various combination vaccines by SmithKline Beecham. More recently, a number of recombinant therapeutic proteins produced in various animal cell lines have gained marketing approval. Chinese hamster ovary (CHO) cells have become popular recombinant production systems, as have baby hamster ludney (BHK) cell lines. Patterns of glycosylation associated with recombinant glycoprotein biopharmaceuticals produced in such systems resemble most closely the native glycosylation pattern when the protein is produced naturally in the body.

The production of recombinant therapeutic proteins in the milk of transgenic animals has also gained much publicity over the last few years. A variety of therapeutically proteins, including tissue plasminogen activator, al-antitrypsin, interleulun 2 and factor IX have been produced in this matter. It is likely that therapeutic proteins produced in such systems will gain regulatory approval within the next few years.

Upstream Processing

After its initial construction, the recombinant producer cell line is thoroughly characterized and its genetic stability verified. The cell line is then used to construct a ‘master’ and ‘worlung’ cell bank system. Initial stages of upstream processing invariably involve lab-scale culture of the contents of a single vial from the working cell bank. Thus, in turn, if is used to inoculate a larger volume of media which (after cell growth) is, in turn, used to inoculate the production scale bioreactor. The scale of fermentation depends upon the level of production required, but generally production scale bioreactors would vary in capacity from one thousand liters to several tens of thousands of liters [1].

Downstream Processing

All biopharmaceutical products must be exhaustively purified with the purpose of removing virtually all contaminants from the product stream. Such contaminants include proteins (related or unrelated to the protein product), DNA, pyrogens, viral particles and microorganisms.

Downstream processing is initiated by recovery of the crude protein product from the fermentation media (if produced extracellular) or cell paste (if produced intracellular). It is next subjected to high resolution chromatographic purification. Generally, at least three different chromatographic steps (e.g. ion-exchange, gel filtration, hydrophobic interaction chromatography or affinity chromatography) are employed, yielding a product which is 98-99% pure. While chromatographic fractionation is designed to remove contaminant proteins from the protein of interest, several chromatographic steps are also quite effective in removing additional potential contaminants from the product stream, Gel filtration chromatography, for example, is usually quite effective in removing any contaminant viruses. After chromatography, excipients are added and the product potency is adjusted by diafiltration by tangential flow as necessary. As therapeutic proteins are heat labile, product sterilization is done by filtration and thus is followed by aseptic filling into final product containers. Although some products may be marketed in liquid format, most are freeze dried. Freeze dried products generally are more stable, exhibiting a longer shelf life than analogous liquid formulations.

Downstream Sampling

Through the downstream process the product is being monitoring in different steps to determine the efficiency of the process step, product quality and to verify that the product is not contaminated maintained at the higher quality standards.

The actual downstream sampling process is performed using a dedicated valve installed at the process tank, illustrated in Figure 1. Before transferring the product sample from the tank to the corresponding sterile bottle, the bottles are sterilized in an autoclaved machine and the sample port is sterilized with a Steam in place (SIP) method. At the beginning of the collection step, product is purged and then collected.

The biological technician perform the aliquot process in a Bio Safety Cabinet (BSC) the quantity collected in the sterile sample bottle is specified in the Production Control Record (PCR) based on the test to be performed. These sampling process steps make the method to be time and product consuming.

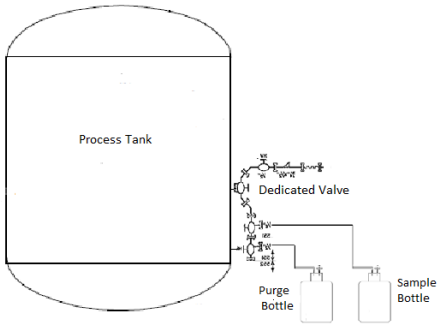

Figure 1
Actual Sampling Process

LEAN SIX SIGMA

The history of Six Sigma is well documented. In brief, it started at Motorola in the late 80s in order to address the company's chronic problems of meeting customer expectations in a cost-effective manner. Instead of thinking of quality as an inspection problem conducted after the fact, it was initiated at the front end of the process and continued throughout the manufacturing process. Each improvement project was organized into the four phases:

- **Measure (M)** - identify what your customers want or need and assess how you are failing to fulfill their expectations.
- **Analyze (A)** - identify the internal causes of the problems.
- **Improve (I)** - make changes to the product or service to improve it.
- **Control (C)** - put signoffs or monitoring programs in place to ensure the improvements continue.

Larry Bossidy, the CEO of AlliedSignal and ex-GE executive, brought success stories to the attention of Jack Welch, the CEO of GE. Jack took to the program completely and applied it across all of GE. Promotions were "frozen" throughout the company until everyone received training. When Jeff Immelt took over as CEO, in early September of 2001, he repeated GE's emphasis on using Six Sigma to achieve a companywide customer focus and individual career success.

Something that is impressive about the program at GE is how it continues to expand to all parts of the business where customer contact is made. Instead of being a program of "Inspected by No. 73", it evolved over the years to become an efficient system of business process improvement with customer focus and solid financial benefit to the company. Senior business leaders at GE must have received Six Sigma training and completed a number of projects before advancing in their careers. Largely owing to the initial failure of the initial projects to deliver the expected financial impact, GE quickly added an extra phase to define and manage the improvement project. The Define, Measure, Improve, Analyze, and Control (DMAIC) structure has now become an accepted standard for Six Sigma project execution and management [3].

DMAIC

The project will be divided into five phases (See Figure 2) following the DMAIC tool (Define, Measure, Analyze, Improve and Control).

Figure 2
DMAIC Process Steps

The DMAIC methodology can be described as follows;

Define (D) - Define the goals of the improvement activity. At the top level the goals will be the strategic objectives of the organization, such as a higher ROI (Return of Investment) or market share. At the operations level, a goal might be to increase the throughput of a production department. At the project level goals might be to reduce the defect level and increase throughput. Apply data mining methods to identify potential improvement opportunities.

Measure (M) - Measure the existing system. Establish valid and reliable metrics to help monitor progress towards the goal(s) defined at the previous step. Begin by determining the current baseline. Use exploratory and descriptive data analysis to help you understand the data.

Analyze (A) - Analyze the system to identify ways to eliminate the gap between the current performance of the system or process and the desired goal. Apply statistical tools to guide the analysis.

Improve (I) - Improve the system. Be creative in finding new ways to do things better, cheaper, or faster. Use project management and other planning and management tools to implement the new approach. Use statistical methods to validate the improvement.

Control (C) - Control the new system. Institutionalize the improved system by modifying compensation and incentive systems, policies, procedures, MRP, budgets, operating instructions and other management systems. You may wish to utilize systems such as ISO 9000 to assure that documentation is correct.

METHODOLOGY

The methodology used for this project was DMAIC [2]. The DMAIC define phase identified the characterization and improve needs for the initial conditions of Product X10 during the sampling process.

Define Phase

This phase includes the introduction and methodology which had been identified. Nevertheless, the define phase required a brainstorming step in where the opportunity to optimize the sampling process for Product X10 was identified. Product X10 sampling process was found with opportunities for improvement based on recent re-sampling and samples contamination events at company PR Biotechnology Solutions Company downstream operation. The sampling process is very vital, since it shows how Product X10 final standards parameters as product pH, conductivity, bioburden, and endotoxin are achieved.

Measure Phase

During this phase the parameters to be considered will be mechanical, assembly time and sample sterility of Product X10, which consist on pH, conductivity (µS/cm), bioburden (cfu/mL), endotoxin (EU/mL).

Two engineering runs will be conducted under this project. One will be using media as a solution since it will be a worst case scenario to test sample sterility. The second will be conducted using the Product X10 as a solution. The time involved in the sampling device installation will be recorded. After the devices installation on the process tank to be use an SIP will be performed. The tank will be filled with the required solution and will be held close with the agitator on for 5 days. Everyday samples will be taking using the two in-processes sampling devices (MN and GSD) and the dedicated valve as a control sample. The samples will go to the laboratory for endotoxin and bioburden

analysis. The results of the analysis will be recorded and analyzed.

Analyze Phase

Using the gathered information from the experiment, the behavior of the solution to be use in the engineering runs as pH, conductivity bioburden and endotoxin during the sampling process will be evaluated from the process data and test results data collected.

Improve Phase

During this phase data analyzed will be used to determine if process changes can be implemented or recommended to improve sampling process and reduce assignable causes. Process changes and recommendations will be focused on mistake proofing. In addition the technical group will recommend any process cycle time reduction if feasible, based on the facts that Product X10 can achieve specification values.

Control Phase

This phase will not be pursued as part of the project due to implementation timeline limitations.

RESULTS AND DISCUSSION

This chapter summarized all the findings observed during the development of this project. The results will be presented following the previously presented methodology.

The two engineering studies were executed for 5 days using the established process parameters pH, conductivity, endotoxin and bioburden as acceptance criteria. The results are shown on Figures 3, 4, 5, 6 and Tables 1, 2 and 3.

Table 1
Endotoxin and Bioburden TSB Media 30g/Kg Results

Engineering Study Run :	1				
Solution:	TSB Media 30g/Kg				
Day	1	2	3	4	5
Endotoxin (<5 Eu /mL)	<5	<5	<5	<5	<5
Bioburden 9 (0 cfu/ ≤100 mL)	0	0	0	0	0

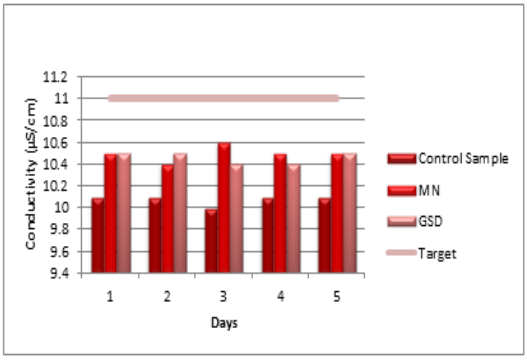

Figure 3
Conductivity TSB Media 30g/Kg Results

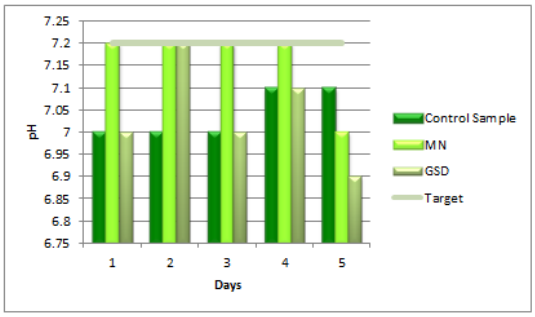

Figure 4
pH TSB Media 30g/Kg Results

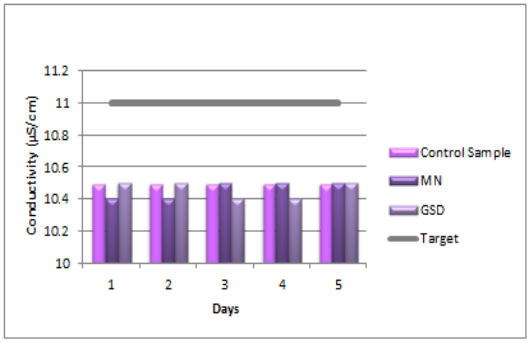

Figure 5
Conductivity Product X10 Results

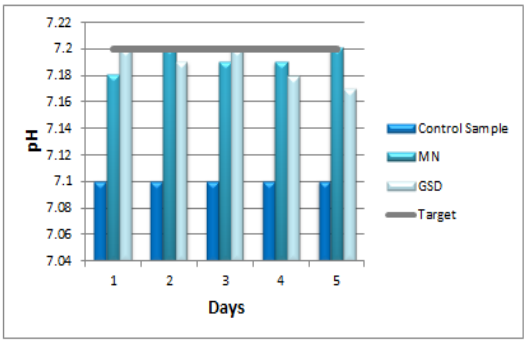

Figure 6
pH Product X10 Results

Table 2
Endotoxin and Bioburden TSB Media 30g/Kg Results

Engineering Study Run :	2				
Solution:	Product X10				
Day	1	2	3	4	5
Endotoxin (<5 Eu /mL)	<5	<5	<5	<5	<5
Bioburden 9 (0 cfu/ ≤100 mL)	0	0	0	0	0

On both runs, the results meet the acceptance criteria for the established process parameters in comparison with the control sample as an actual sampling method for the 2 sample devices. The evaluation of the mechanical installation performed for GSD device and MN devices in terms of the installation time was very similar for both devices. Therefore, there is no significant difference between the devices installation time period.

CONCLUSION

After evaluating the information gathered from the engineering studies PR Biotechnology Solutions Company technical support group concluded that both sampling devices MN and GSD are capable to collect the in process samples from process tank without affecting the established process parameters or compromising the sterility of the sample. Therefore, both devices are recommended to be used and will result in a cost and cycle time reduction to the manufacturing operations of PR Biotechnology Solutions Company.

The new downstream sampling process will reduce currently cycle time of Product X10 by 87 % since the sampling process take around 15 hrs for every sample and after this sampling initiative improvement implementation it will take approximately 2 hrs. Therefore, based in these facts, PR Biotechnology Solutions Company technical group performed a cost reduction analysis. The total cost reduction with this strategy will be $85,960 immediately the new propose change is implemented.

Table 3
Downstream Sampling Process Cost Analysis

	Actual Process	After Sampling Improvement Implementation
Sampling Process Time	15 Hrs	2 Hrs
Product Line Reduction	-	$ 56,100.97
Autoclave material cost reduction	-	$ 29,859.36
	Total	$ 85,960.33

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