Improvements to Perfusion Skids 66501/66502/66503 Operations Used for Enbrel Product in AML-06

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Abstract *— Amgen AML-06 Drug Product Plant is a multi-product facility where therapies for chronic diseases are manufactured. Its systems are designed for the continuous improvement of the processes, in order to comply with the supply requirements for medications.*

The project to be worked will impact the manufacturing process specifically for the Perfusion process of Enbrel product. The perfusion process is carried out in Bioreactor N-1, a previous step to Bioreactor N where the protein is finally produced. The team will be working with symptoms in the Perfusion equipment, which cause the process time to exceed the validated limits.

To achieve this, we will use the DMAIC methodology, with the intention of improving an implemented process. The goal is that we can implement improvements to the process and that the time of the process is within the established ranges, and thus not impact the integrity of the cells or the schedule of the lots. To meet the goal, it is necessary to achieve a reduction of at least 6% of the processing time.

Key terms *— logical control, mechanical seal, perfusion, SIP*

PROJECT STATEMENT

The Manufacturing Department of the AML-6 Plant raised the concern because the metrics of the Perfusion process in the Cell Culture area, reported high downtime hours during the manufacture of the product Enbrel. The engineering team was activated with the intention of investigating the root causes that cause the high downtime hours. The recipes that are run during the process (Perfusion, CIP and SIP), are stopped by alarms that are triggered from the perfusion skids 66501/66502/66502 and cleaning skid 40508 operations used for Enbrel product. Figure 1 shows one of the Perfusion Skid (66501), where improvements will be implemented.

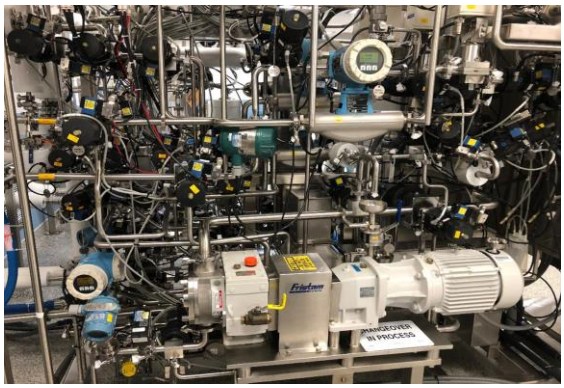

Figure 1
Perfusion Skid 66501

Project Description

During this project we will investigate problems associated with the Perfusion process for Enbrel product. Using the DMAIC methodology, we will implement improvements for this process that is a very important and crucial step in the overall manufacture of Enbrel.

Project Objectives

The objective of this project is to identify the root causes for the downtime hours and solutions or improvements to be applied to the Perfusion process. Achieve that the skids operations run consistently in order to maintain an optimum Perfusion process for the manufacture of Enbrel product.

Project Contributions

The improvements to the skid's operations of the Perfusion process, will avoid the downtime

generated, causing a delay in the process or that the batch being discarded for not complying with the required residence time. Achieve that the cells go to the final bio-reactor in a consistent manner. Also, this research can be applied to the perfusion skids used for the others product manufactured in AML-6 or other sites of Amgen.

BACKGROUND

Perfusion

Perfusion technology was first introduced in the late 1980s to boost the low product concentrations obtained from the early cell lines used for biopharmaceutical manufacturing. Dramatic increases in titers for batch and fed-batch cell-culture processes over the next two decades largely eliminated the need for perfusion and interest waned. A key driver today is the production of unstable proteins that require low residence times in the bioreactor [1].

In Perfusion process, high cell numbers are sustained for much longer periods by constantly feeding fresh media and removing spent media while the cells remain in culture (figure 2). With this approach, optimum conditions for growth and production are maintained by supplying the appropriate nutrients and removing toxic waste products. Because the product is also regularly removed and separated from the waste products that can cause degradation, perfusion is highly beneficial for biologic APIs that are unstable under production conditions [1].

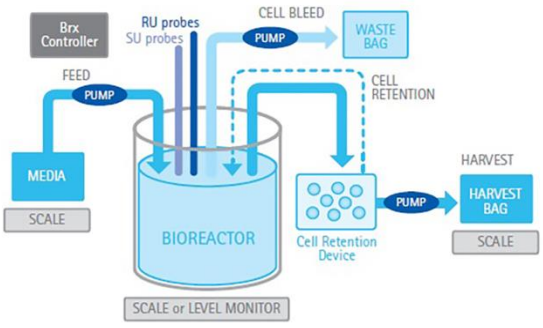

Figure 2
Typical Perfusion process in bio-reactors

Properly designed perfusion processes can significantly increase volumetric productivities (grams/L of bioreactor working volume per day such that the bioreactor scale can be significantly reduced, which facilitates adoption of disposable technologies (figure 3). The result is an increase in operational and capacity flexibility, reduced capital and operational costs, and increased speed (facility readiness and manufacturing operation) [1].

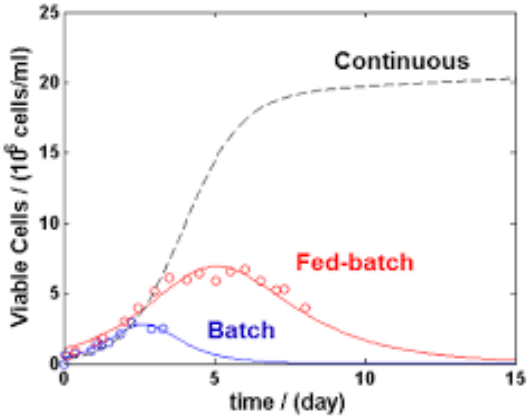

Figure 3
Graph illustrating how is the performance of Batch, Fed-Batch and Continuous processing achieving the required cell density. Perfusion was designed as a Continuous processing.

SIP

SIP (Steam in Place) is a timed sterilization of the upstream and downstream biopharmaceutical production train using clean steam. It is part of a 5-step sanitization routine that occurs after every production batch, and follows the final rinse after CIP (Clean in Place). SIP ensures that every square inch of the production train that come in contact with drug substance or drug product is sterilized to ensure that there is no microbiological activity in the system [2].

The clean steam used in Amgen is made of WFI (Water for Injection) and is circulated through all of the process tubing during this stage, and enters large vessels through spay balls embedded in the vessel ceiling. SIP is a temperature validated process, where the minimum sterilization regimen requires the injection of clean steam for at least 30 minutes after they reach a minimum temperature of 121°C (figure 4).

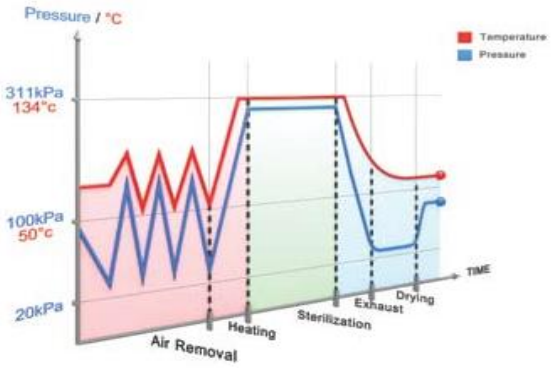

Figure 4
Typical SIP (Steam in Place) process.

For the three phases that compose the sterilization cycles (Pre-Conditioning, Exposure and Post-Conditioning), six factors are particularly critical to assure successful steam sterilization: Time, Temperature. Moisture, Direct steam contact, Air removal and Drying [3].

Logic Control

A logical control program is a set of conditional statements describing the response of a controller to different inputs. A controller is a computer used to automate industrial processes. Process engineers use control logic to tell the controller in a process how to react to all inputs from sensors with an appropriate response to maintain normal functioning of the process. Control logic (sometimes called process logic) is based on simple logic principles governed by statements such as IF X, THEN Y, ELSE Z yet can be used to describe a wide range of complex relationships in a process. Although different controllers and processes use different programming languages, the concepts of control logic apply, and the conditions expressed in a logical control program can be adapted to any language. Bio-chemical processes evaluate input values from the process against set values to determine the necessary actions to keep the process running smoothly and safely [4].

Logical controls (IF, THEN, ELSE, and WHILE) compare a value from a sensor to a set standard for the value to evaluate the variable as True/False in order to dictate an appropriate response for the physical system. The control program for a chemical process contains many statements describing the responses of valves, pumps, and other equipment to sensors such as flow and temperature sensors. The responses described by the system can be discrete, such as an on/off switch, or can be continuous, such as opening a valve between 0 and 100%. The goal of a control program is to maintain the values monitored by the sensors at an acceptable level for process operation considering factors like product quality, safety, and physical limitations of the equipment. In addition, to describing the normal activity of the process, a control program also describes how the process will initialize at the start of each day and how the controller will respond to an emergency outside of the normal operating conditions of the system. Unlike a linear computer program, logic programs are continuously monitoring and responding without a specific order. Before constructing logical control programs, it is important to understand the conditional statements, such as IF-THEN and WHILE statements, that govern process logic [4].

Pump Mechanical Seal

The demands on shaft seals in pumps designed for sterile and sanitary applications differ entirely from those made on other seals. Often the seal needs to comply with standards and regulations. In some instances, the seal materials must comply with guidelines for cleanability and resistance to the pumped media and be capable of CIP, cleaning-in place, and SIP, sterilization-in-place. In addition, low roughness values and electro polished surfaces, marked yellow, are required on medium side components. Special attention must be paid to the elastomer components of the shaft seal. Elastomer components must withstand the pumped media and temperatures in the cleaning processes. The purpose of these requirements is to ensure that all shaft seal surfaces in contact with the pumped media can be cleaned [5] (figure 5).

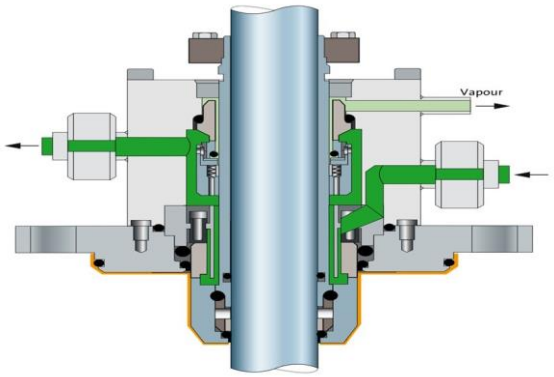

Figure 5
Example of complex sanitary agitator seal subject to the highest sterilization and cleanability requirements. The barrier fluid (green) can be steam condensate. Surfaces marked with yellow are electro-polished. Secondary seals on medium side have been modified, leaving no gaps.

METHODOLOGY

The project methodology to be used in this research will be the DMAIC process. DMAIC is an acronym for Define, Measure, Analyze, Improve, Control. DMAIC is the process improvement methodology of Six Sigma that's used for improving existing processes [6].

Define

Define phase is when teams move from very basic information about a process or problem to the knowledge and organization necessary to enter measure and subsequent other phases with a successful foundation. In the define phase, teams set rules, create a charter that will govern efforts moving forward, identify stakeholders and customers, define a process through process mapping, and prepare for a define tollgate before entering the measure phase [6].

Measure

One of the important tasks of the measure phase is the data collection. For this, the team will have a strong understanding of current process performance. The team should define a process specific metric where possible and gather historical data regarding that metric, so they have something to compare future data against to prove that improvements were made [6].

Analyze

This third phase of the Six Sigma methodology needs that the measure phase be a strong one. The Analyze phase of DMAIC helps project teams identify problems in the production process that cause product defects. Is loaded with tools to help spot the problems in the production process and to determine if these problems are the root causes of defects. Process mapping is used to identify bottlenecks, repetition, and delays. It also helps to define the process boundaries, process ownership, and process responsibilities [6].

A popular method for this phase is the Fishbone Diagram. This diagram lets teams to concentrate on a brainstorming process in a logical way, visualize the information to identify priorities and trends [6].

Improve

During the improve phase of a DMAIC project, the team brainstorm possible solutions for the root causes identified in the analyze phase and rank those solutions according to costs, how effective the solution would be and how likely the solution could be implemented. Teams pilot solutions through beta tests or small roll outs, collect data in the solution and verify that the solution is working as expected via statistical analysis. If the solution is a confident one to address the problem, the team plans and implements a full rollout of the solution [6].

Control

During the control phase, teams build monitors that let them ensure the process continues to work successfully after changes are implemented across the regular business process. Also, the transition the process back to the process owner is worked. Appropriate documentation via a control plan and education regarding tools such as control charts might be necessary to ensure business teams can

maintain a process and identify when it's out of control and needs remediation [6].

RESULTS AND DISCUSSION

This chapter presents the problems analysis, improvements suggested, and the results obtained using the DMAIC Methodology.

Define

In this step, the observations found in the perfusion system will be discussed in detail. The itinerary to follow for the evaluation of the problems, the proposal of the improvements and the date of their implementation will also be proposed. It was decided to build a multidisciplinary team to work on the improvements and their implementations in a short period of time. The team will be made up of the following positions: Engineering (System Owner and Automation), Process Owner, Quality, Manufacturing and Maintenance.

The Perfusion process have a validated duration of 120 hrs. ± 12 hrs. For the last 10 batches, the average duration time was 140 hours. This is unacceptable due to compromise the complete bio-reactor train schedule. This situation causes that the bio-reactor N-1 (Perfusion), cannot be ready for the next batch on time due to others preparation activities like cleaning and sanitization.

The improvement proposal only impact perfusion skids 66501/66502/66502 and cleaning skid 40508 operations used for Enbrel product in AML06.The following are the problems detected in the perfusion skid operation:

- Pump Mechanical Seal damage.
- Batch microbial contamination.
- Leaks observed in the flexible hoses.

A schedule was created (table 1) with the intention of establishing the necessary activities and their duration.

Table 1

Schedule proposed for the DMAIC exercise

Task Name	Duration	Start Date	Finish Date	Completed
Define	5 days	03/23/2020	03/27/2020	√
Measure	5 days	03/30/2020	04/03/2020	√
Analyze	5 days	04/06/2020	04/10/2020	√
Improvement	25 days	04/13/2020	05/08/2020	√
Control	5 days	05/11/2020	05/15/2020	√

Measure

In this phase, the team was dedicated to obtain data of the Process Time for the last 10 batch manufactured. As shown in table 2, only two batches complied with the specified time. The hours above of 132 hours are considered downtime hours. Some of this batches were discarded due to the cells were adversely affected. Figure 6 shows how was the trending for the batches

Table 2

Process Time per Batch

Batch	Time (hrs)
1	155
2	139
3	145
4	125
5	134
6	146
7	142
8	125
9	152
10	137

Analyze

Next to establish the problems that lead to downtime hours, we use the Fishbone Diagram in order to identify the root causes (figure 7).

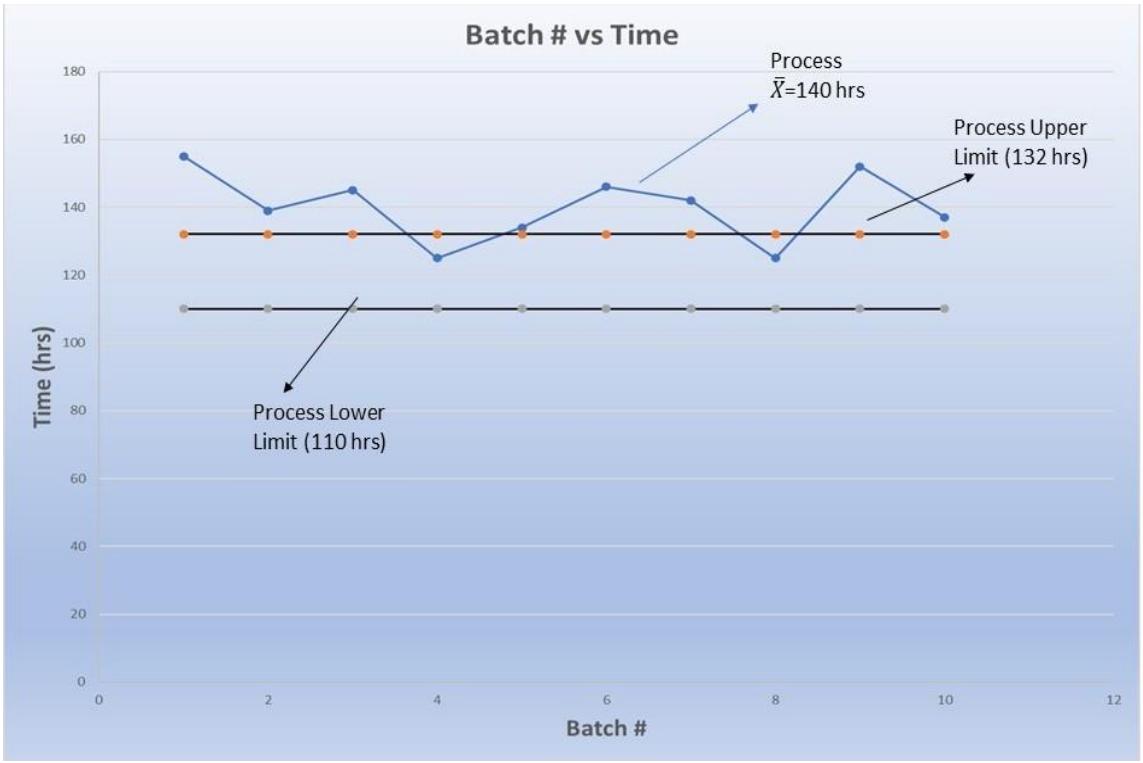

Figure 6

Graph illustrating the process trend for the last 10 batches manufactured

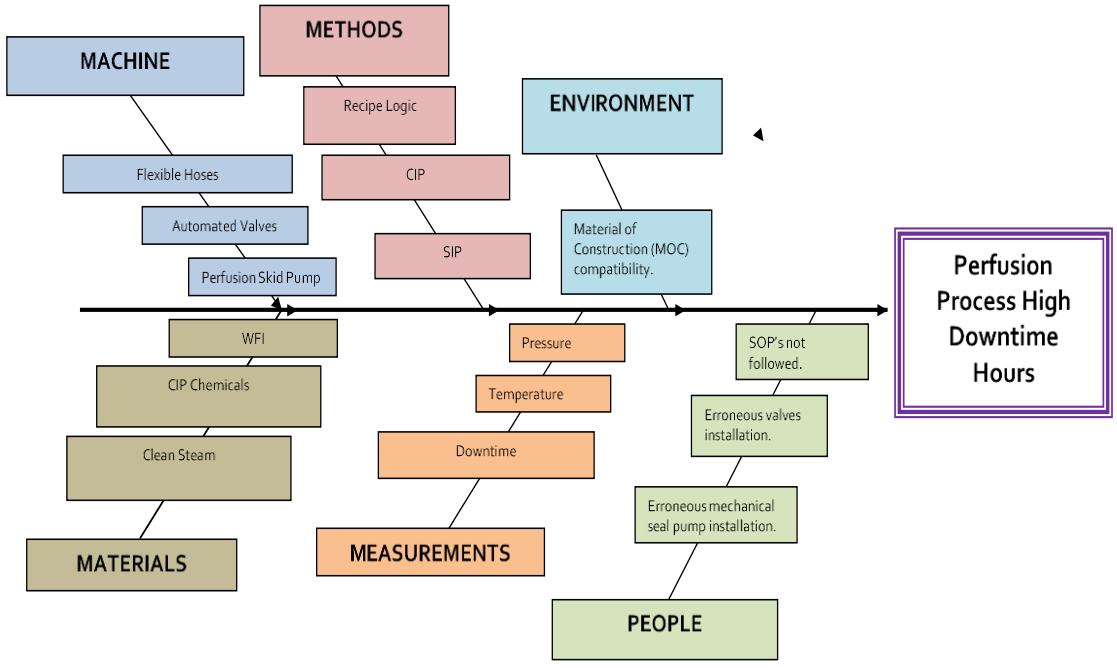

Figure 7

Fishbone Diagram for Root Causes determination

From the exercise, the following root causes arise for the downtime situation:

- The system integrity has been affected due to the pump mechanical seal have been damaged by exposition to high temperature operations during the CIP, Water for Injection (WFI) flush and Process stages. Currently, Perfusion pumps do not have a temperature interlock during CIP, Water for Injection (WFI) flush and Process stages. If the pump is operated at high temperatures, the pump seal could be damage and impact the integrity of the system.
- The system integrity has been affected due to the pressure operation for CIP, Water for Injection (WFI) flush and Process stages. Higher pressures than the pressure of the mechanical seal side, can cause an integrity breach. Currently, Perfusion pumps do not have a pressure interlock during the Process stage. For some instances, the pump was operated with a higher pressure in the process side than in the pump seal pressure side, resulting in an integrity breach of the pump seal and hence in a product contamination.
- Pump mechanical seal have been impacted by vacuum conditions generated during the SIP process. This create an integrity breach and that the products are contaminated. Currently, after the SIP process is complete, Perfusion pump seal inlet valve XV-703 closes during cooldown, which causes the steam to collapse and creating vacuum conditions which impact the integrity of the pump seal. As well, after the SIP process is complete, the steam supply inlet valve XV-710 closes and the steam supply line of the Perfusion skid cools down and non sanitary tri-way ball valve XV-700 is not constantly steamed, resulting that this be a contamination focus.
- Currently during perfusion skid SIP cooldown, bioreactor valve 5423X-XV-220 stays open until the temperature reaches 25°C and cooldown is complete. As well, during perfusion skid transfer in line SIP cooldown, valve 5423X-XV-901 and transfer panel 76042 steam traps valves (XV-224, XV-228, XV-229) and 76044 steam traps valves (XV-224, XV 228, XV-229) stays open until temperature reaches approximately 30 °C and cooldown is complete.
- Sagging was observed in the flexible hose of 66501-FH-05 of perfusion skid 66501 and 66502-FH-05 of perfusion skid 66502. A downward bulge in these hoses produces leaks at the clamps connections and promote a small amount of condensate accumulation and possible microbial growth.

Improve

After evaluating all the possible scenarios and root causes, the team concluded that the proposed improvements will avoid the downtime hours. All improvements were managed through the change control system.

The first improvement of the project is the implementation of a temperature interlock condition for Perfusion pumps. The proposed change is to create a new temperature interlock condition for Perfusion pumps 66501P01, 66502P01 and 66503P01 to alarm and put recipes into hold when Perfusion pump seal exit temperature reaches to 50°C. This allows manufacturing associates to verify the pump's heat exchanger temperatures and make arrangements.

The second improvement is the implementation of a pressure interlock condition for Perfusion pumps. The proposed change is to create a new pressure interlock condition for Perfusion pumps 66501P01, 66502P01 and 66503P01 to alarm and close drain valve XV-262 to maintain seal pressure, when Perfusion pump pressure is ≤10 psig higher than process pressure.

The third improvement to be implemented is to modify the Perfusion skid steam supply line after SIP is complete. This change will eliminate vacuum conditions in the pump seal after the SIP process is complete and maintain the steaming of the perfusion steam supply line up to tri-way ball valve XV-700. The proposed change is to modify Perfusion skid SIP logic to maintain steam supply

up to tri-way valve XV-700 and eliminate vacuum conditions in the perfusion pump seal, after the SIP is complete. These changes are:

- Maintain open XV-710, close XV-701 and put XV-700 to trap.
- Close XV-262 and maintain open XV-703 during cooldown.
- Activate EM Seal after cooldown.

The fourth improvement to be implemented is to modify the cooldown logic of perfusion SIP and transfer inline SIP, to close valves and traps while these are still expose to high temperatures. The proposed change is to modify the recipe value PAR_COOLDOWN_TEMP from 25°C to 100°C in the perfusion skid SIP cooldown, and to modify the recipe value PAR_INOCULUM_LINE_COOLDOWN_TIME from 25 minutes to 1 minute in the perfusion skid transfer in line SIP cooldown.

The fifth improvement to be implemented is to replace flexible hoses (FH-66501-01, FH-66502-01 and FH-66503-01) located in steam supply line of perfusion skid by a stainless-steel spool piece. The proposed change is to replace flexible hoses (FH-66501-01, FH-66502-01 and FH-66503-01) by a stainless-steel spool piece to maintain adequate and firm piping slope.

Control

Change Controls records were closed and systems were returned to manufacturing. Manufacturing procedures (SOPs) were revised to include instructions to monitoring the process. Data of temperature and pressure will be documented in official manufacturing documents (Forms). Alarms were included in the Process Control System (PCS), to alert the manufacturing associates.

Data was obtained for the first 5 batches after the improvement's implementation. It is important to note that the first 5 batches were free of microbial contamination. This data is presented in table 3 and figure 8.

Table 3
Process Time per batch after improvements implementation

Batch	Time (hrs)
1	126
2	125
3	128
4	121
5	127

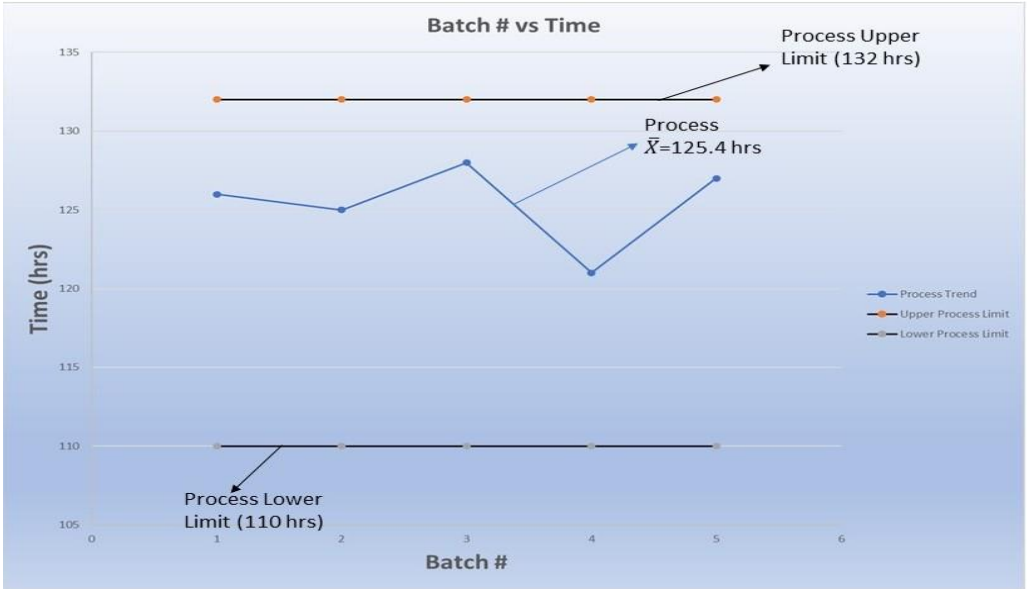

Figure 8
Graph illustrating the process trend for the first 5 batches manufactured after the improvements implemented

CONCLUSIONS

The DMAIC methodology results very effective to analyze the problem, investigate the root causes and establish the improvements needed. Five enhancements were implemented to improve the perfusion process in a short period of time. In summary, the following improvements were performed:

- Sterilization for all components of the skid equipment were optimized.
- The logic of the SIP recipes and the transfer of the medium to the Bio-reactor were optimized.
- The possibility of leaking and contamination was decreased by replacing the flexible hose.
- Interlocks were established to protect the mechanical seal of the pump and thus avoid contamination and equipment down situations.

After analyzing the data of the first 5 batches after the improvements, we can see that the average process time was 125.4 hrs. Fourteen (14.6 hrs.) less for an equivalent of a time reduction of a 10.43%. This achievement allowed that the batches not be discarded and the equipment for the perfusion process to be ready to receive the next batch after the required cleaning and sanitizing processes.

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