Antibody Reduction Mitigation during Harvest from Cell-Free Protein Synthesis

Allison Dwyer Karl Wessendorf Bob Kiss Jimmy Zawada Bryan Dransart
Sutro Biopharma, South San Francisco, CA

Background

- XpressCF(+)™ allows for the efficient production of non-natural amino acid containing antibodies in less than 24 hours.
- Temperature, pH and dissolved oxygen (DO) are controlled parameters during the protein synthesis step but previously DO was not controlled during harvest.

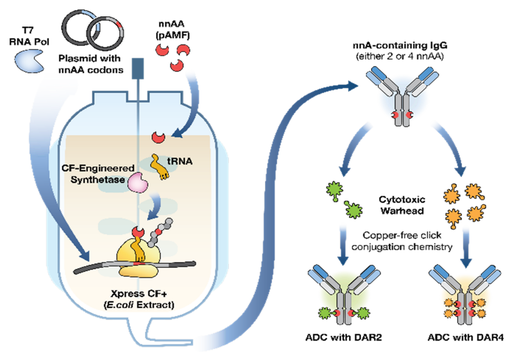

Figure 1: Sutro Biopharma's XpressCF(+)™ technology to express unique ADC's

Problem Statement and Objective

- Significant reduction of disulfide bonds was observed during harvest of a pilot plant production run (200L cell-free) due to extended processing time without dissolved oxygen control. Bench-scale runs with faster processing times did not show disulfide reduction. The sensitivity of the disulfides to processing time demonstrated a need for further development of the process for robust scale-up.

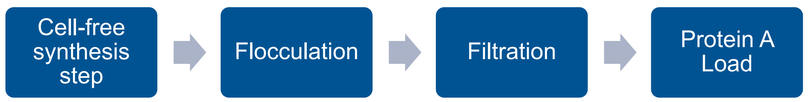

Figure 2: Process flow diagram from synthesis step through harvest

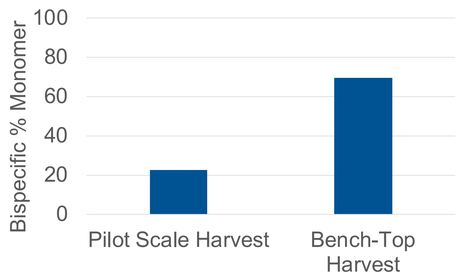

Figure 3: Process flow diagram from synthesis step through harvest

Design

- The first experiment was a dissolved oxygen versus oxidation-reduction potential scout in 250 mL STR containing cell-free. Determining the relationship between the two is important to understand any potential differences in environment during the expression step and cell-free flocculation and harvest.
- The second experiment was designed based on previously reported mitigation strategies for CHO cell culture harvest. Historical harvest procedures include chilling the reactor, sparging air for DO control, lowering pH, and/or adding certain chemical inhibitors[1,2]
- Figure 4 depicts the parameters that were tested on retained cell-free material from the pilot plant production batch that suffered disulfide reduction.

Parameter	Original Parameters	Proposed Changes
Temperature	25°C	20°C
DO	Off	20%
pH	7.0	6.5

Figure 4: Proposed parameter changes during flocculation and harvest

Results

DO vs ORP

- At the end of the cell-free protein synthesis step, the ORP is between -150 and -180 mV and the dissolved oxygen is set at 80%. This range became the target mV ORP to reach during harvest because it is known that the antibody is assembled in this environment.
- The previous harvest condition (no DO control) demonstrated a highly reductive environment at approximately -350 mV.
- The DO vs ORP scout showed that 20% DO is a safe target to match the pre-harvest cell-free ORP.

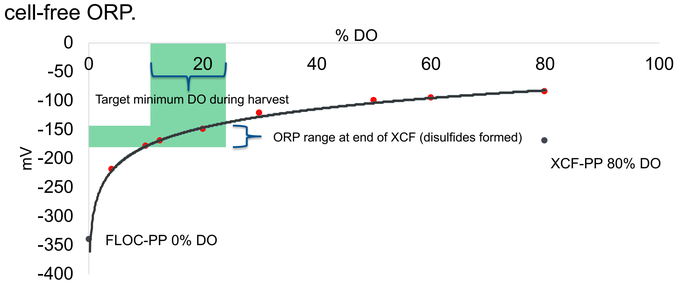

Figure 5: Dissolved oxygen vs. ORP graph

Results continued

New Harvest Parameters

- Previously frozen material from the pilot plant production batch (200L #1) was thawed and harvested using the proposed changes. The harvest was held for 6 hours to mimic a large scale process.

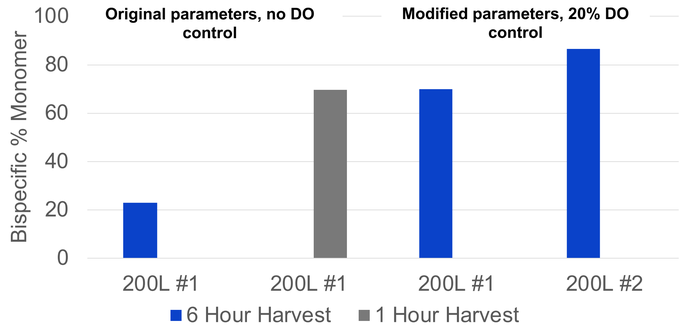

Figure 6: Intact bispecific monomer analysis before and after implementation of cell-free harvest condition changes.

Summary/Conclusions

- As dissolved oxygen is increased, the ORP mV levels in XpressCF(+)™ increased as expected for a more oxidative environment. The target range for harvest ORP is between -150 to -180 mV to match the ORP at the end of XpressCF+™.
- Implementation of disulfide bond reduction mitigation strategies were successful in preserving intact monomer.
- Decreasing the temperature slows metabolic processes, dissolved oxygen control maintains the ORP at the target level, and acidification provides protection for disulfide bonds.
- Applying the new harvest conditions to the batch of cell-free that suffered from major reduction was able to preserve the assembly.

References

1. Mun M, Khoo S, et al. Biotechnology and Bioengineering. Apr 2015,112(4), 734-742.
2. Schmidt M, et al. Biotechnology and Bioengineering. Jun 2010, 106(3), 452-461.

SUTRO BIOPHARMA