A Small-Scale Model for Studying Resin Interactions with Chemical Sanitants

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Introduction

- In integrated, continuous antibody manufacturing, the downstream capture step is effectively part of the upstream process
- Single-use components help extend the sterile barrier from the bioreactor to the capture step
- Gamma-irradiated columns are costly and not conducive to low-cost antibody manufacturing

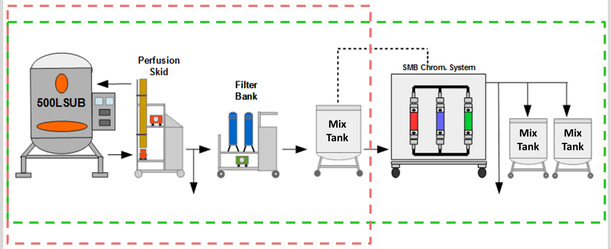


Figure 1. The effective sterile barrier extending beyond perfusion skid (red box) and through the capture system (green box) in an integrated, continuous antibody manufacturing process.

Chemical sanitants can be used to treat reusable materials such as chromatography resin and column housings

- **Peracetic acid (PAA)** is an oxidizing agent with rapid antimicrobial and sporicidal activity
- PAA solutions consist of a mix of Peracetic Acid, Acetic Acid, and Hydrogen Peroxide (H_2O_2)
- PAA and H_2O_2 act synergistically to eliminate bacterial spores, which represent a worst-case contamination scenario[1]
- PAA has been shown to eliminate high concentrations of bacterial endospores completely, whereas high concentrations of NaOH cannot[2]
- Studies have also shown the effect of brief PAA exposure to be minimal on the performance of Protein A columns[2]

- Just had successfully implemented a sanitization procedure for Protein A chromatography capture in 500L productions using a dilute PAA solution
- Just experienced a series of contaminations at large-scale after changing the Protein A resin used in this step

Main Goals

1) **Develop a robust, small-scale model for sanitizing chromatography resins with chemical sanitants**
2) **Use the results of this study to inform a new process-scale column sanitization procedure**

Materials/Methods

Resin Sanitization Method:

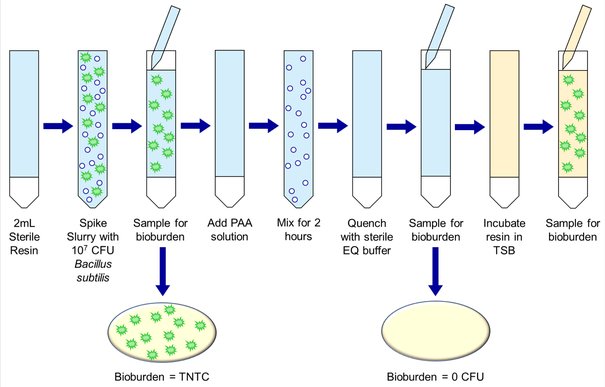


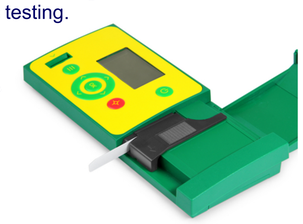
Figure 2. Schematic of small-scale resin sanitization model. This approach allowed for controlled, high-throughput testing of multiple Protein A resins, chemical sanitants, and bacterial spiking conditions.

Spiking Bacteria:

Bacillus subtilis spores were spiked using BioBall® (bioMérieux), which provides a precise number of bacteria. *Bacillus subtilis* spores are often used as model organisms in antimicrobial effectiveness testing.

PAA & H_2O_2 Assay:

Working concentrations of PAA and H_2O_2 in the resin slurries were measured using a Reflectoquant® RQflex® 20 reflectometer (EMD Millipore)



Resins Tested:

Protein A Resin	Matrix/Backbone
ProA - 1* ProA - 4	Agarose
ProA - 2* ProA - 2 Unconjugated Base Beads ProA – 3	Methacrylate

*The contaminations occurred after switching the continuous capture resin from ProA -1 to ProA - 2

Buffers:

Name	Composition
EQ	Neutral pH Buffer
PAA	0.2% PAA (w/v)
PAA/EtOH*	0.2% PAA (w/v), 20% EtOH
TSB	Tryptic Soy Broth

*A PAA/EtOH solution was evaluated based on literature that indicated ethanol may enhance the sporicidal activity of PAA[3]

Results

Three out of Four Resins Tested Could be Sanitized

Protein A Resin	Bioburden (CFU)		
	Before PAA	2hr PAA	2hr PAA/EtOH
ProA - 1	TNTC	0	0
ProA - 2	TNTC	**TNTC**	**~200**
ProA - 2 Base Beads	TNTC	**TNTC**	**TNTC**
ProA - 3	TNTC	0	0
ProA - 4	TNTC	0	0

Table 1. TNTC = Too Numerous to Count. All conditions were tested in replicates of 2 or more. Both negative and positive bioburden results were confirmed by suspending the treated resin samples in TSB for ≥3 days and sampling the TSB for bioburden.

Lack of sanitization of ProA-2 and ProA-2 Beads correlated with lower concentrations of PAA and H_2O_2

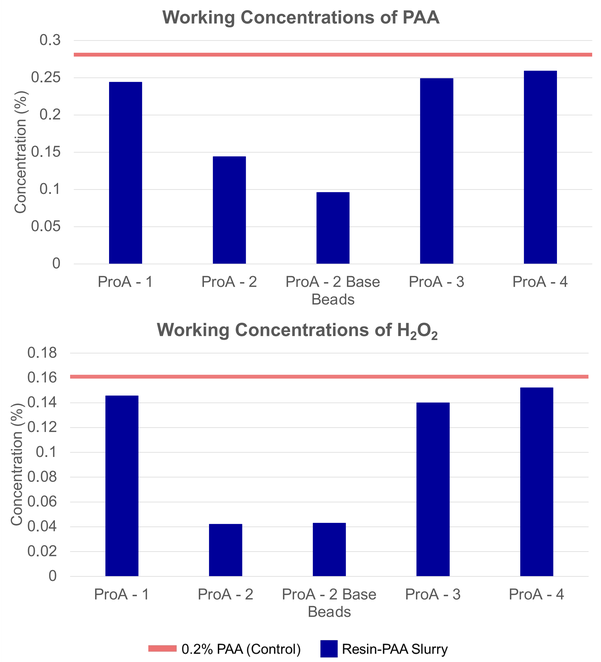


Figure 3. Average working PAA (top) and H_2O_2 (bottom) levels in 0.2% PAA solution and PAA-resin slurries. Working levels are noticeably lower in slurries with ProA - 2 resin compared to other resins. These results were similar in spiked resin slurries and slurries not spiked with bacteria.

Results suggest the resin backbone interacts with and reduces PAA and H_2O_2, resulting in insufficient bacteria kill.

Conclusions

- The apparent interactions between the ProA - 2 base matrix and PAA were identified as a potential cause of the observed contaminations
- These results informed process-scale column sanitization work, and ultimately resulted in a sanitization procedure that successfully enabled a bioburden-free GMP continuous capture process **for 14 days[4]**
- These results emphasize the importance of screening new materials for their compatibility with continuous processes – which includes chemical sanitants and other bioburden reduction methods

Production Day Bioburden (CFU)						
Unit Op	**Day 8**	**Day 10**	**Day 12**	**Day 15**	**Day 19**	**Day 22**
SUB	0	nt	0	0	0	0
SUSV1	0	0	0	0	0	0
ProA-2nd Pass	0	0	0	0	0	0
ProA EL	nt	nt	0	0	0	0
SUSV2	nt	0	0	0	0	0
SUSV3	nt	0	0	0	0	0
SUSV4	nt	0	0	0	0	0
Final Pool	--	--	0	0	0	0

Table 2. nt = not tested. Bioburden results from an integrated continuous run. No bioburden was detected at any sampling point along the process, including the ProA 2nd Pass and ProA Elution pools[4]

Lessons Learned in Sanitization Model Development

- Spike resin with bacterial spores instead of vegetative bacteria, and hold for at least 1 day to mimic a worst-case contamination scenario
- Resuspend treated resin in TSB or other cell culture media for at least 2 days to confirm bioburden assay result
- Conditions tested at small-scale should be verified at process-scale, which includes applying cell culture media to a column for a prolonged period of time to mimic continuous capture conditions

References

1) Leggett, M. J., et al. (2016). Mechanism of sporicidal activity for the synergistic combination of peracetic acid and hydrogen peroxide. *Appl. Environ. Microbiol.*, 82(4), 1035-1039.
2) Application note: Impact of sporicidal agent on MabSelect SuRe Protein A resin lifetime. GE Healthcare, 29262168, Edition AA (2017).
3) Nerandzic, M. M., Sankar C, T., Setlow, P., & Donskey, C. J. (2016). A cumulative spore killing approach: synergistic sporicidal activity of dilute peracetic acid and ethanol at low pH against *Clostridium difficile* and *Bacillus subtilis* spores. In *Open forum infectious diseases* 3(1), ofv206. Oxford University Press.
4) Piper, R. (2020). Protein A column sanitization: Enabling continuous antibody production for GMP manufacturing [PowerPoint Slides].

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