Impact of sporicidal agent on MabSelect SuRe protein A resin lifetime

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Impact of sporicidal agent on MabSelect SuRe™ protein A resin lifetime

Bacterial endospore contamination constitutes a challenge in bioproduction due to their resistance to high concentrations of NaOH, commonly used in sanitization procedures. Hence, there is an interest in evaluating oxidizing agents with sporicidal activities, such as peracetic acid (PAA), in the control of such contaminants. However, many of the protein-based chromatography resins used in purification of biomolecules are sensitive to harsh sanitization conditions. In this application note, the effects of treating MabSelect SuRe protein A affinity resin with 20 mM PAA every fourth process cycle for more than 100 cycles were investigated. The results show that treatment with 20 mM PAA for 30 min or 30 mM PAA for 15 min could be used without significantly affecting mAb recovery or purification performance of the resin, as compared with a control process without PAA treatment. The frequency of PAA treatments, however, should be determined specifically for each mAb process.

Introduction

Bacteria originate from water, soil, air, plants, animals, and humans and can enter manufacturing processes through various routes. In bioproduction, potential sources of contamination are personnel working in the manufacturing facility, equipment, process operations, raw materials, chromatography resins, filter membranes, process gases, and water. An incident is costly, as it typically results in production stop with a subsequent quality assurance investigation of the root cause (1). Ultimately, the facility needs to be sanitized and, in worst case, the whole production lot and the resin discarded. As bacterial contamination can affect the safety and integrity of the bioproduct, the demand for bioburden control from regulatory authorities is also strict. Although the primary goal is to prevent entrance of bacteria by working aseptically, it is also essential to establish efficient sanitization methods that can be used both proactively and in case of a bacterial contamination incident.

Some bacteria can form highly resistant, dormant structures called endospores under conditions unfavorable for growth. When conditions allow, the spores transfer to a vegetative state, enabling microorganism to start propagating. The presence of spore forming bacteria that are resistant to high NaOH concentrations is therefore a challenge in bioproduction, and oxidizing agents efficient in spore reduction are gaining interest for use in industrial process decontamination. The oxidizing agent PAA has shown to be efficient in reduction of bacterial spores. Treatment with 30 mM PAA for 15 min or 20 mM PAA for 30 min resulted in a more than 6 log reduction of *Bacillus subtilis* spores.

MabSelect SuRe is an alkali-stabilized protein A affinity resin. For coupling of its ligand to the base matrix, a cysteine residue is used. Uncoupled, this cysteine residue is completely oxidized to sulfonic acid at low PAA concentrations. However, in-house data shows that when coupled to the base matrix, oxidation of the cysteine residue occurs but the reaction is much slower. When subjected to treatment with 0.1 M PAA for up to 24 h, no effect on the resin binding capacity was observed. A patent application for this sanitization method for affinity chromatography is pending (2). The purpose of the current work was to evaluate the long-term effects of PAA treatment of MabSelect SuRe chromatography resin in a functional lifetime study. To mimic sanitization between batches in a mAb purification process, treatment with 20 mM PAA for 30 min was included in every fourth protein A cycle.

Materials and methods

Bacterial spore challenge test

B. subtilis (ATCC 6633) spores were added to a 50% MabSelect SuRe resin slurry in either 1.0 M NaOH, 1.0 M NaOH + 2% benzyl alcohol (BnOH), 1.0 M NaOH + 40% 2-propanol, 30 mM PAA, or 20 mM PAA to a concentration of about 10^8 CFU/mL. The reducing effect was evaluated after given time intervals by neutralization of the disinfectant.

Table 1. Process conditions used in the MabSelect SuRe purification step

Phase	Buffer	Volume (CV)	Flow velocity (cm/h)	Residence time (min)
Equilibration	Sodium phosphate, 150 mM NaCl, pH 7.4	4	343	3.5
Sample load	30 mg mAb/mL resin (1.73 g mAb/L culture feed)	16.13	200	6
Wash 1:1	Sodium phosphate, 150 mM NaCl, pH 7.4	1.5	200	6
Wash 1:2	Sodium phosphate, 150 mM NaCl, pH 7.4	2.5	343	3.5
Wash 2	Sodium phosphate, 1.0 M NaCl, pH 7.4	4	343	3.5
Wash 3	Sodium acetate, pH 5.5	4	343	3.5
Elution	Sodium acetate, pH 3.7	1.5	120	10
Strip or sanitization	Citric acid monohydrate, pH 2.1 or 20 mM peracetic acid, pH 3.0	3	120 (30 min contact time)	10
Re-equilibration 1	Sodium phosphate, 150 mM NaCl, pH 7.4	2	120	10
Cleaning in place (CIP)	0.1 M NaOH	3	120 (30 min contact time)	10
Re-equilibration 2:1	Sodium phosphate, 150 mM NaCl, pH 7.4	1.5	120	10
Re-equilibration 2:2	Sodium phosphate, 150 mM NaCl, pH 7.4	3.5	343	3.5

Lifetime study

Sample preparation

Clarified Chinese hamster ovary (CHO) cell culture supernatant, containing mAb (IgG1) at a concentration of 1.73 g/L (courtesy of Josh Goldstein, Janssen R&D, LLC, Malvern, PA, USA), was filtered using an ULTA™ Pure HC 0.2 µm filter before use.

Experimental setup

Tricorn™ 5/200 columns were packed with MabSelect SuRe resin to a bed height of 20 cm, corresponding to a column volume (CV) of 4 mL. The column was connected to an ÄKTA™ system. The mAb capture step outlined in Table 1 was performed over 108 cycles. Every fourth cycle, the acidic strip was replaced by treatment with 20 mM PAA at a contact time of 30 min. Fresh PAA solution was prepared at the day of use. As control, the same process was performed without PAA treatment over 52 cycles. Instead, the acidic strip was performed each cycle. Sample was loaded at 30 mg mAb/mL resin, corresponding to 50% of the dynamic binding capacity (DBC) at 10% breakthrough (Q_{B10}), at a residence time of 6 min.

Analyses

DBC was evaluated by frontal analysis (FA) using a polyclonal human IgG (Gammanorm) before start of the lifetime study, and then after 20, 40, 50, 72, and 108 cycles. In the FA, IgG was diluted in equilibration buffer to 2.0 mg/mL. First, the IgG solution was injected, by-passing the column, to obtain a maximum UV absorbance value at 280 nm. After equilibration of the MabSelect SuRe column, IgG was loaded until breakthrough, using a flow rate corresponding to a residence time of 6 min. Unbound IgG was washed off with equilibration buffer, and bound IgG was eluted with citric acid monohydrate, pH 2.1. Q_{B10} was determined from the breakthrough curve, using a standardized spreadsheet.

To determine mAb recovery during the lifetime study, selected eluates were analyzed for mAb content using the Biacore™ T200 system. In addition, the sanitization and strip peaks were analyzed by liquid chromatography-mass spectrometry (LC-MS).

Host cell protein (HCP) concentration in the product pool was analyzed using commercially available anti-CHO HCP antibodies (Cygnus Technologies) and Gyrolab™ workstation. Leakage of MabSelect SuRe ligand was determined using a commercially available ELISA kit (Repligen Corp.).

Results

B. subtilis spore challenge test

As shown from the *B. subtilis* spore challenge test, 1.0 M NaOH is not sufficient for reduction of spores to below 100 CFU /mL, not even after an extended contact time of 24 h (Fig 1). Treatment with 1.0 M NaOH and 2% benzyl alcohol (BnOH) resulted in a slightly improved spore reduction, and by treating the resin with 1.0 M NaOH and 40% 2-propanol at a contact time of 4 h, a > 6.2 log reduction of spores was achieved. With PAA, a > 6.2 log reduction of *B. subtilis* spores were observed already after a 15 min treatment with 30 mM PAA or after a 30 min treatment with 20 mM PAA.

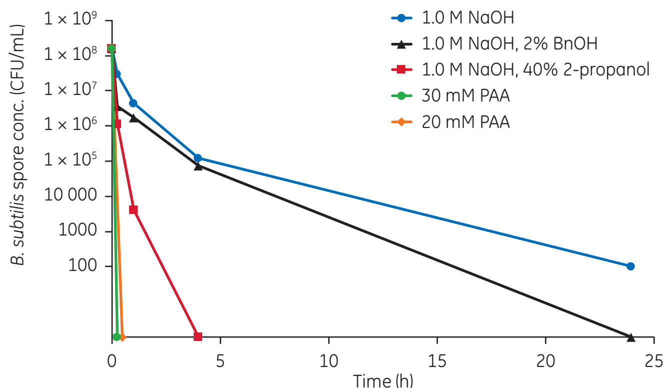

Fig 1. *B. subtilis* spore challenge test in a 50% MabSelect SuRe resin slurry.

MabSelect SuRe resin lifetime study

In a previous study, MabSelect SuRe resin was subjected to treatment with 30 mM PAA over 10 protein A capture cycles (5 h total contact time). The results showed no significant decrease in DBC or impact on purification performance. After 20 cycles, however, including PAA treatment in each cycle, the DBC decreased by 15%–20%. For this study, it was therefore decided to evaluate MabSelect SuRe resin lifetime when including treatments with 20 mM PAA every fourth cycle. As control, the same process was performed without including the PAA treatment step.

HCP reduction was comparable between processes, with HCP levels in the range of about 300 to 500 ppm for the process including PAA treatment and 500 to 600 ppm for the control process (Fig 2). Although low, ligand leakage was higher with PAA treatments, with ligand levels in the range of 8 to 23 ppm, as compared with less than 10 ppm for the control process (Fig 3). However, although a 20% decrease in Q_{B10} over 50 cycles in the process including PAA treatment, mAb recovery was still maintained above 90% (Fig 4 and 5). As comparison, Q_{B10} decreased by 10% over the 52 cycles of the control process, with mAb recovery maintained above 95%. After 108 cycles, Q_{B10} had decreased by 40% in the process including PAA treatment, while recovery was still above 80%.

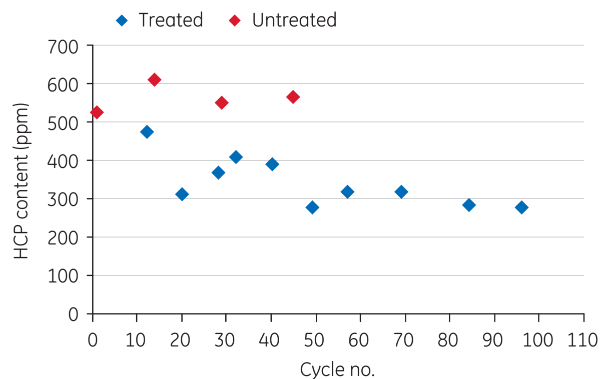

Fig 2. HCP content in process including a PAA treatment step and in process without PAA treatment step.

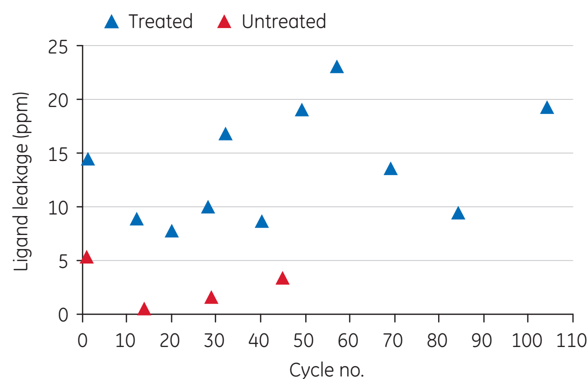

Fig 3. Ligand leakage in process including a PAA treatment step and in process without PAA treatment step.

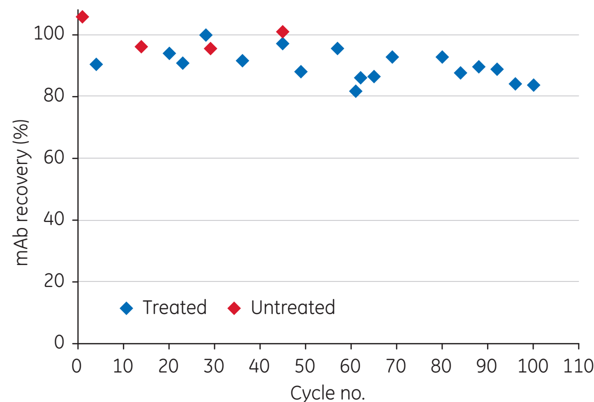

Fig 4. mAb recovery over process cycles including PAA treatment and without the PAA treatment step.

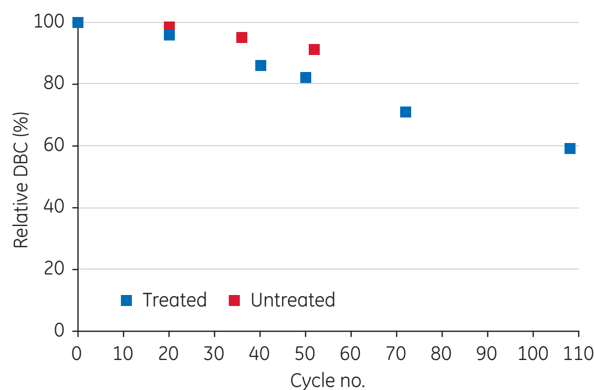

Fig 5. Relative DBC over process cycles including PAA treatment and without the PAA treatment step.

In the process including PAA treatment, the initial elution pool volume of 1 CV increased over time, but tended to level out at approximately 1.5 CV after 80 cycles (Fig 6). The strip peaks also increased over time, which can be related to the broadening of the elution peaks. An overlay of the column elution, strip, and CIP peaks are shown in Figure 7. Analysis of the sanitization and strip peaks showed presence of IgG heavy and light chains, however, no ligand was observed in these peaks.

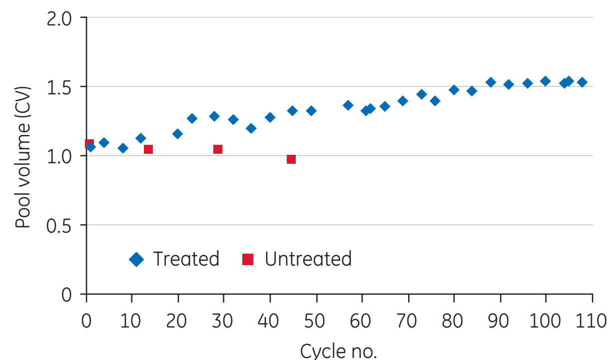

Fig 6. Elution pool volumes over process cycles including PAA treatment and without the PAA treatment step.

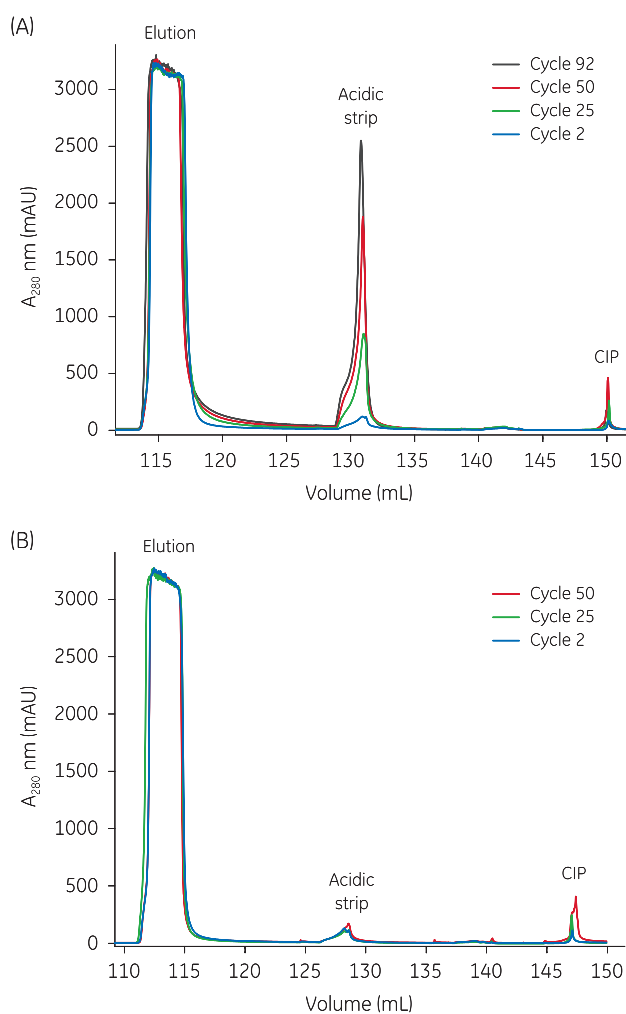

Fig 7. Overlay of elution, acidic strip (citric acid), and CIP peaks over process cycles (A) including PAA treatment and (B) without the PAA treatment step.

Considerations for PAA use

PAA is a strong oxidizing agent and care should be taken during handling of this chemical. Considerations should also be made for compatibility of equipment to be used in processes including PAA treatment. Information from literature on PAA compatibility of materials used in wetted parts of chromatography process hardware and single-use components from GE Healthcare intended for use in biomanufacturing processes are given in Table 2. For borosilicate glass, PEEK, and TPX, no information on PAA compatibility was available. However, as these materials were found to be compatible with hydrogen peroxide, another strong oxidizer, it is assumed that the materials are also compatible with PAA. As PA, TPE, and EPDM can be affected by PAA to some extent, experimental data was generated on tensile strength and shrinking for EPDM; melt index, hardness, and density for TPE; tensile strength for PA; and stickiness for silicone to confirm the compatibility of these material with exposure to up to 100 mM PAA for up to 24 h. Based on these findings, materials used in wetted parts of AxiChrom™ and BPG columns as well as ReadyToProcess™ columns and ÄKTA ready flow kits are considered compatible with PAA under conditions used in this work (20 mM for 30 min or 30 mM for 15 min). It should be noted, however, that polyurethane, used in the stand of BPG columns, is not compatible with PAA. Hence, exposure of the stand to PAA should be avoided during column use.

Table 2. PAA compatibility of materials used in wetted parts of manually packed AxiChrom and BPG columns as well as in prepacked ReadyToProcess columns and single-use ÄKTA ready flow kits

Materials	Comments
Acrylic Polytetrafluoroethylene (PTFE) Polypropylene (PP) Polyoxymethylene (POM) Fluoroelastomer (FKM) Stainless steel Fluoroethenepropylene (FEP) Fluorocarbon rubber (FPM) Ultra-high molecular weight polyethylene (UHMWPE) Tygon™ 2275 Polystyrene (PS) Polyethylene (PE) Polyvinylidene difluoride (PVDF)	Compatible with PAA, no significant effect noted.
Borosilicate glass Polymethylpentene (TPX) Polyether ether ketone (PEEK)	No information on PAA compatibility available. Compatible with hydrogen peroxide (H_2O_2).
Polyamide (PA) Thermoplastic elastomer (TPE) Ethylene propylene diene monomer (EPDM) Silicone	Some effect observed, especially at high PAA concentrations. Additional tests confirm compatibility with 100 mM PAA for exposure up to 24 h.

Conclusions

In this study, the long-term effects of PAA treatment on MabSelect SuRe resin performance was investigated. The resin was subjected to 20 mM PAA in a total of 28 treatments with a total contact time of 14 h over 108 protein A process cycles. Although an additional capacity loss of 10% after 50 cycles, compared with the untreated resin, no major negative impact of PAA treatment was observed on mAb recovery and HCP reduction. Considering the big impact that a bacterial contamination incident might have on biomanufacturing, our results show that PAA could still be a viable option for use in industrial process decontamination without major impact on mAb purification performance in the capture step using MabSelect SuRe resin. Based on our results, treatment with 20 mM PAA for 30 min or 30 mM PAA for 15 min could be applied. However, frequency of PAA treatment should be determined case by case, and column lifetime should be evaluated for each individual mAb process. The final decision on sanitization protocol will depend on the safety margin during mAb load (here, 50% of Q_{B10} was used) and the number of cycles that the protein A resin will be used for before discarded, that is, the useful lifetime. As a general guideline, sanitization with PAA could be performed before column use, between campaigns, and in case of a bioburden incident for remediation of the resin, that is, two to three times during the resin lifetime. Before applying PAA treatment, consideration of PAA compatibility of process equipment, such as column hardware and single-use components, should also be made.

Disclaimer

The results and conclusions presented in this application note are valid for this specific study only. Other study conditions could have significant impact on the outcome. Method for sanitization using PAA should be decided case by case and lifetime of the packed column should be evaluated for each process. The overall finding is that MabSelect SuRe resin can be treated with 20 mM PAA for 30 min or with 30 mM PAA for 15 min under conditions used in this work without major impact on resin performance. Sanitization with PAA should not replace CIP with NaOH. In this case study, sanitization with PAA was included as a replacement of an acidic strip in order not to introduce an additional phase in the protein A step. It could be beneficial to regenerate (strip) and clean the MabSelect SuRe resin before sanitization to reduce the risk of fouling.

The information contained herein is not representative of any specific claims or any relevant environment, health, and safety laws and regulations, including use authorization, product registration or application licensing, or similar legal requirements.

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Ordering information

Product	Size	Product code
MabSelect SuRe	200 mL	17543802
Tricorn 5/200	5 mm i.d.	28406412
ULTA Pure HC 0.6/0.2 μm filter	0.1 m^2	KMP-HC9204TT

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