Biotechnology and Bioengineering

WILEY

BIOTECHNOLOGY AND BIOENGINEERING

ARTICLE OPEN ACCESS

Multiple Use of Regenerated Depth Filters in Antibody Purification Processes

Bernhard Spensberger | Ferdinand Stueckler | Marc Pompiati | Christoph Feistl | Thorsten Lemm | Roberto Falkenstein

Roche Diagnostics GmbH, Penzberg, Germany

Correspondence: Roberto Falkenstein (roberto.falkenstein@roche.com)

Received: 14 October 2024 | **Revised:** 10 February 2025 | **Accepted:** 12 February 2025

Keywords: antibody | carbon footprint | depth filter reuse | downstream purification | impurity removal | regeneration | sustainability

ABSTRACT

During the manufacturing of therapeutic antibodies, disposable depth filters are used after affinity chromatography to remove haze and process-related impurities such as host cell proteins (HCP) and DNA known as critical quality attributes. The present study reports on the regeneration of depth filters allowing their reuse for at least 10 times while retaining sufficiently high clarification capacity. Three filter types were evaluated including standard cellulose-based and fully synthetic matrix materials using acidic or alkaline solutions in alternating cycles of loading and regeneration. Both alkaline and acidic solutions were effective, however, overall acidic regeneration of the filter material appeared superior for multiple use. This was especially evident for the silica-containing XOSP filter, where HCP and DNA were almost completely removed and remained low over 10 applications. Simultaneously preserved product quality indicated a high resistance of the filter matrix toward regeneration. These unexpected findings offer improved flexibility for available filter capacity in downstream processing along with ecologic advantages over the single use applications. Regarding the carbon footprint of the filtration process, calculated potential savings by a factor of four can be achieved, mainly accounting for reduced plastic waste. Therefore, depth filter reuse supports sustainability and carbon dioxide reduction during production processes.

1 | Introduction

The manufacturing of cell culture-based biopharmaceutical such as monoclonal antibodies (mAbs) requires extensive purification procedures to achieve satisfactory yield and purity (Liu et al. 2010; Shukla et al. 2007) and is increasingly challenged by high cell densities and mAb titers implicating high contents of process-related impurities (Singh et al. 2013). Among various clarification technologies, depth filtration methods may be located at several steps within the downstream purification process (Singh et al. 2016). Their application in primary clarification usually after centrifugation mainly removes cell debris and insoluble material from the cell culture harvest, thereby protecting subsequent chromatography columns or sterile filter from plugging and preserving high performance and lifespan of resins (Chandler and Zydney 2005). Further downstream, for example, after mAb capture, depth filtration can be employed for secondary clarification and haze removal applications (Kandula et al. 2009; Hogwood et al. 2013) for instance, in cases where low pH adjustment and neutralization of eluates may result in precipitates (Yigzaw et al. 2006; Schreffler et al. 2015). Furthermore, depth filtration can be flexibly implemented before or after viral inactivation, ion exchange chromatography, viral filtration, and ultrafiltration, thereby achieving improved matrix and column lifetime for the polishing steps as well (Iskra et al. 2013).

Beside these beneficial properties, depth filters are known to achieve considerable level of impurity clearance, especially of process-related components such as HCP (Yigzaw et al. 2006; Khanal et al. 2018) and DNA, (Charlton et al. 1999; Khanal et al. 2019) and even the removal of product-related impurities,

like high and low molecular weight forms, has been reported (Nguyen et al. 2019; Yu et al. 2019). Early reduction of the impurity load usually facilitates the performance of following chromatography steps (Koehnlein et al. 2024).

Generally, depth filters utilize a multilayer matrix of materials capable of building a gradient in density and pore size (Chandler and Zydney 2005). In addition to size exclusion, particle and impurity retention is known to involve adsorption through hydrophobic, ionic, and other interactions, like hydrogen bonding (Charlton et al. 1999; Khanal et al. 2018; Yu et al. 2019). The different filter materials used correspond to different depth filter types. Commonly, the porous matrix is made up of cellulose fibers supplemented with a filter aid to increase the surface area and a stabilizing binder. For instance, negatively charged diatomaceous earth (DE) used as filter aid is well suited to bind positively charged and hydrophobic proteins (Nejatishahidein et al. 2020). The naturally derived components may, however, exhibit certain performance variability and can be the source of organic and ionic extractables (Nguyen et al. 2019; Holstein et al. 2021). In contrast, a recently introduced all-synthetic filtration media with more defined filter specifications is suggested to offer higher consistency of filter composition and functional properties and was shown to improve impurity removal regarding HCP and aggregates in comparison to standard filters (Nguyen et al. 2019; Parau et al. 2022). Like DE, the silica used as a filter aid in this matrix is suitable for adsorbing positively charged HCP by electrostatic and hydrophobic interactions (Ghose et al. 2004).

Depth filters are usually applied in single use and represent an example for a typical single use technology (Budzinski et al. 2022). Beside the anticipation of constant clearance properties, disposable filters allow continuous processing and are favorable in view of continual production systems (Lalor et al. 2019), because a system shutdown for cleaning in place can be avoided (Grönberg et al. 2011). However, waste and costs usually increase with single use applications compared to multiple use. In addition to overall cost-reduction, there is a strong need for ecological saving of resources (Lalor et al. 2019; Whitford et al. 2022).

The fouling mechanisms occurring during depth filter use may include pore blockage, cake formation and pore constriction (Parau et al. 2022). Therefore, efficient regeneration is required to eliminate the filtered impurities and to restore matrix conditions and clearance characteristics that are as effective as those of unused filters. Alkaline regeneration using sodium hydroxide has become the standard for cleaning and sanitizing chromatography columns due to sterilizing and bacteria-inactivating properties (Burgoyne et al. 1993). However, chromatography media using a protein ligand or based on silica or glass may be sensitive to sodium hydroxide. For instance, sodium hydroxide treatment can induce hydrolysis of siloxane bonds in the silica matrix (Claessens et al. 1996). Materials sensitive to alkaline solutions may be alternatively subjected to acidic treatment which is another well-known approach to regenerate matrices and resins (Berggrund et al. 1994).

The present study investigated whether the cleaning and retention performance of depth filters applied as disposables in secondary clarification can be restored by means of regeneration to a grade which enables multiple use of a single filter. For this purpose, several experimental series with multiple cycles of filter use and regeneration were performed evaluating alkaline and acidic regeneration solutions with different filter types. Surprisingly, both regeneration approaches were effective in restoring HCP and DNA removal over multiple cycles without affecting the quality of the monomer product. Overall, the high clearance capacity retained especially with acidic regeneration demonstrated that depth filters can be reused at least 10 times while maintaining performance comparable to single use. Based on these unexpected results, an estimate of the corresponding ecological savings potential was prepared.

2 | Materials and Methods

2.1 | Load Material

The humanized IgG1 antibody had a standard format and was produced in Chinese hamster ovary cells. Filtered cell culture supernatant was applied to a Protein A Mab Select SuRe column (GE healthcare) and washed with buffer. mAbs elution at low pH was followed by neutralization. Pooled mAb eluate was conditioned to pH 5.5 and stored at −80°C.

2.2 | Depth Filters

The PDD1 SUPRAcap-50 depth filter (Pall Corporation) is composed of cellulose fibers, DE, and perlite with a filter area of 22 cm^2.

The Zeta Plus Biocap VR02 depth filter (Zeta GmbH) is composed of a cellulose fiber matrix and charged surface groups resulting from ionic charge modifications with a filter area of 25 cm^2.

The Millistak+ HC Pro XOSP depth filter (Merck Millipore) is composed of a synthetic polyacrylic fiber matrix and silica as filter aid. The filter with a filter area of 23 cm^2 is intended for secondary clarification applications (Nguyen et al. 2019).

2.3 | Incubation and Regeneration Solutions

The buffer used for equilibration and washing was composed of 150 mM acetic acid/tris, pH 5.5. The alkaline regeneration solution consisted of 1.0 M sodium hydroxide. Acidic regeneration was performed with two solutions, either with 500 mM phosphoric acid or with 167 mM acetic acid, 300 mM phosphoric acid.

2.4 | Experimental Design

Filtration experiments were conducted with an Äkta Avant 150 chromatographic system (Cytiva), where the filter was installed to the column valve. Pressure, pH value, conductivity, OD280 were monitored, and the applied volume was regulated by a

sample pump. The filter operating delta-pressure limit of 2.8 was not exceeded.

Filters were conditioned with water and equilibration buffer (each with 100 L/m^2 and a flow of 10.0 mL/min) before use. For loading, feed flows in the range of 200 L/m^2/h (XOSP: 200 L/m^2/h, PDD1: 191-200 L/m^2/h, VR02: 184 L/m^2/h) were applied with a mass throughput of approximately 600 g/m^2 (599–690 g/m^2). Volume throughput was always adjusted to a calculated loading flow of 7.67 mL/min. The filter flowthrough was collected for OD280 values exceeding 0.5 AU. After the loading was completed, the filter was flushed with equilibration buffer to wash out the remaining protein solution and flowthrough was collected for 70 L/m^2.

The filters were prepared for the next filtration use by conditioning with water and equilibration buffer (see above). This represented the procedure for filter regeneration of the reference experiments and was performed before regeneration with alkaline and acidic solutions.

In case of alkaline treatment, the filter was preincubated after initial conditioning using 70 L/m^2 of 1 M sodium hydroxide (flow of 7.67 mL/min) with subsequent incubation for 4 h before use. Filter regeneration was performed after protein washout using 1 M sodium hydroxide for approximately 30 min. The regeneration solution was removed with water (100 L/m^2). In total, the filter was exposed to values above pH 10 for approximately 50 min. To prepare the filter for the next filtration use, conditioning with equilibration buffer was performed as described above.

Acidic regeneration of filters was conducted after protein washout using either 500 mM phosphoric acid or a milder solution of 167 mM acetic acid, 300 mM phosphoric acid (flow of 7.67 mL/min) for approximately 30 min. The regeneration solution was removed with water (100 L/m^2). In total, the filter was exposed to values below pH 2 for approximately 50 min. To prepare the filter for the next filtration use, conditioning with equilibration buffer was performed as described above.

The procedure of alternating product filtration and regeneration was repeated for up to nine cycles resulting in 10 filter uses (see overview of the filter regeneration process in Figure 1 for large scale and Supporting Information: Figure S1 for small scale experiments). In total, protein amounts up to approximately 6000 g/m^2 (10 × 600 g/m^2) were applied to the filter. Obtained filtration pools were kept at −80°C until analysis.

2.5 | Analytics of Process-Related Impurities HCP and DNA

The residual HCP content in the samples was determined using the CHO HCP electrochemiluminescent immunoassay on Cobas e 801 immunoassay analyzer (Roche Diagnostics, detection limit 2 ng/mL, quantification limit 7.5 ng/mL, linear measuring range up to 1000 ng/mL) and DNA originating from host cells was measured using an automated quantitative polymerase chain reaction method performed in the 96-well format by the FLOW FLEX system (Roche Diagnostics, detection limit

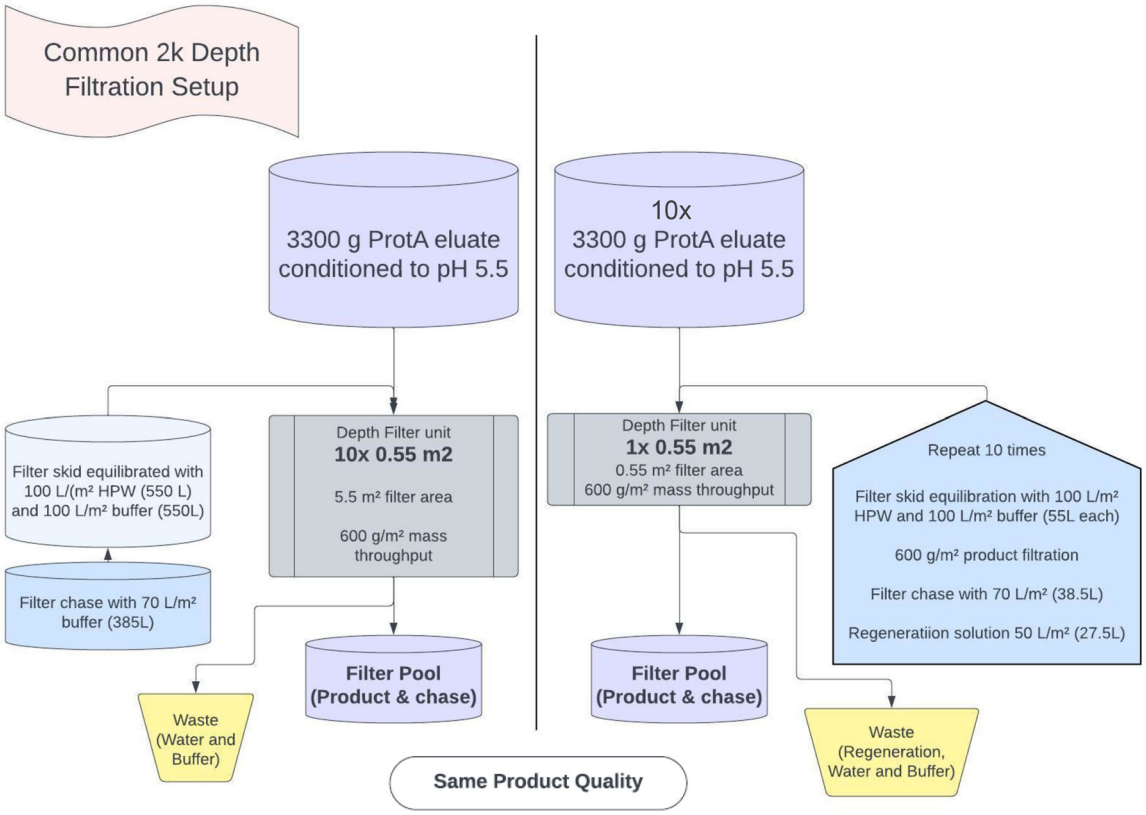

FIGURE 1 | Overview of the experimental setup comparing the application of 10 depth filter units in single use with the tenfold use of a single filter unit. The consumption of materials and solutions was calculated for a 2000-L filtration scale (2k) corresponding to approximately 3300 g protein obtained from Protein A (ProtA) eluate.

0.4 pg/mL, quantification limit 4 pg/mL, linear measuring range up to 4000 pg/mL) as previously described in detail by Koehnlein et al. 2023. In the present study, the results for both impurities were normalized to the protein concentration in mg/ml. For HCP, the results obtained in ng/ml were expressed in ppm. For DNA, the results obtained in pg/ml were expressed in ppb.

2.6 | Main Peak Analysis

Main peak analysis determining the level of the antibody monomer under native conditions was performed by size exclusion high performance liquid chromatography (SE-HPLC) as described by Koehnlein et al. 2023.

2.7 | Sustainability Assessment

The calculation of carbon dioxide emissions for the comparison of the two procedures (10 single use filters vs. tenfold use of a single filter) was performed for processing a 2k batch (2000 L) as shown in Figure 1. For all buffer materials and water, emission factors from the EcoInvent data base (version 3.9.1; 2023) were used.

All emission factors accounted for production, transportation and disposal of materials (incineration of filters, water and buffer solution disposal to sewage) including energy consumption. Based on these specifications, the carbon footprint (kg CO_2 equivalent) per L used solutions was assessed as shown in Table 1.

The energy required for the extra process time resulting from filter reuse was not considered in the calculation. Calculations confirmed that it was negligible compared to the overall energy balance and CO_2 footprint of the process. In addition, the resulting CO_2 equivalents are variable because they depend on the share of renewable energy in the power supply.

For the filters, no emission factors were available. Therefore, emission factors were estimated using the weight of the material. Since the filters are mainly composed of plastic, an average of EcoInvent emission factor for plastic species was used. To account for the complexity in manufacturing GMP-ready filter material, the plastic factor was multiplied by four. This estimation resulted in 12.5 kg CO_2 equivalents per kg filter and 62.5 kg CO_2 equivalents per filter unit used for a 2k batch.

3 | Results and Discussion

3.1 | Comparison of Alkaline and Acidic Regeneration

The possibility for restoring the clearance capacity of filters applied for secondary clarification of mAb eluates obtained from affinity-capture was evaluated in experimental series using alkaline (1 M sodium hydroxide) and acidic (0.5 M phosphoric acid) regeneration solutions. The effects of these regeneration solutions on the filter performance were studied in comparison to the buffer solution used for washing and equilibration (150 mM acetic acid/tris, pH 5.5). Sodium hydroxide was additionally applied for initial filter pre-incubation in accordance with GMP guidelines regarding sterilization and endotoxin inactivation in a closed system. In each series, a total of ten filter uses was examined by executing cycles alternating protein loading and filtration with filter regeneration. For each use, the filter performance regarding the clearance of HCP and DNA impurities was measured and the product quality of the filtered mAb was assessed by main peak analysis.

3.1.1 | Filter With Cellulose Fiber Matrix

The PDD1 filter was used as representative for a standard cellulose filter containing DE and perlite as filter aid. Starting at high HCP and DNA levels in the load, this filter removed virtually all impurity content upon first use (Figure 2A,B). After second filter use (including one regeneration cycle), a slight increase in the HCP level was detected in the flow-through of the buffer reference and the acidic regeneration with values lower than 5%, while the HCP content of the alkaline procedure remained low (Figure 2A). After third use, the buffer reference showed a pronounced increase (21%) which continued during following uses and finally reached a kind of plateau with values in the range of 50% of the load. In contrast, the HCP levels obtained by continuous acidic regeneration showed only minor additional increment finally reaching 6.5% of the load value. The levels for alkaline regeneration, however, remained below 2% until seven filter

TABLE 1 | Carbon dioxide emissions (kg CO_2 equivalents) determined for the solutions used.

Solution	kg CO_2 equivalent/L	Volume/2k (L)	kg CO_2 equivalent/2k
Water	0.010	1000	10.0
Equilibration buffer 150 mM acetic acid pH 5.5	0.030	1700 Equilibration + chase	51.0
Acidic regeneration 500 mM phosphatic acid	0.089	500	44.5
Acidic regeneration 167 mM acetic acid, 300 mM phosphatic acid	0.080	500	40.0
Alkaline regeneration 1 M sodium hydroxide	0.113	500	56.5

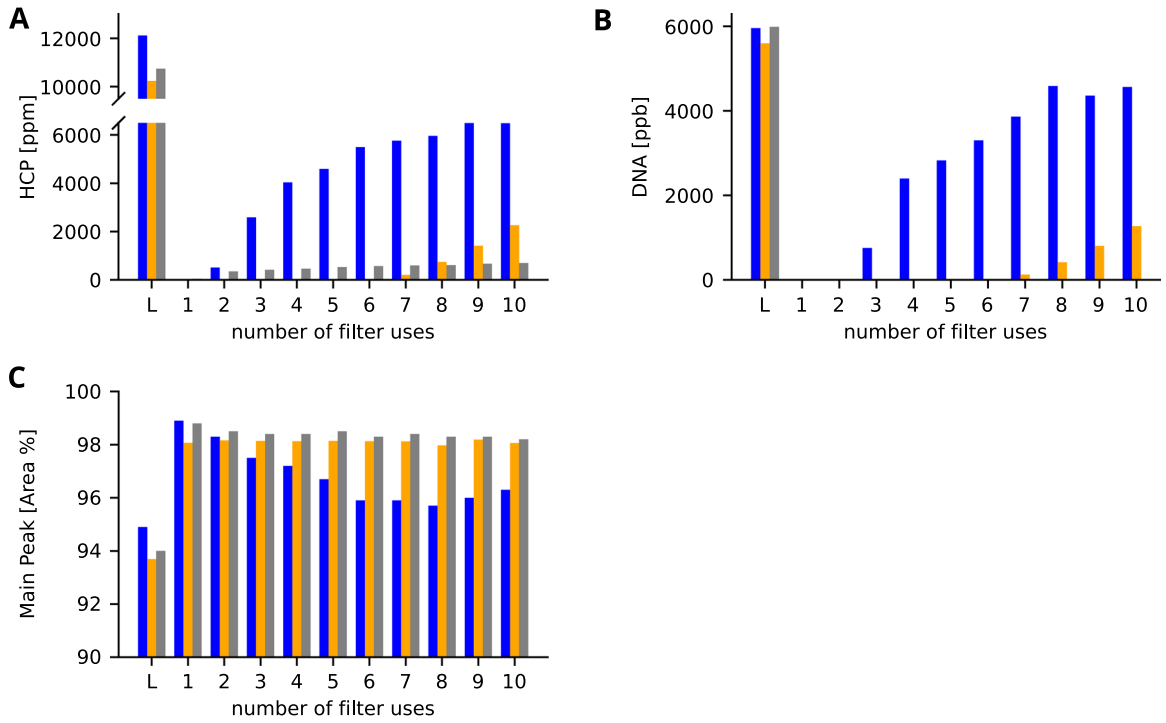

FIGURE 2 | Comparison of alkaline and acidic regeneration for the reuse of the cellulose-based depth filter PDD1 (blue, equilibration buffer 150 mM acetic acid/tris pH 5.5; yellow, alkaline solution 1 M sodium hydroxide; gray, acidic solution 500 mM phosphoric acid) displaying (A) HCP removal, (B) DNA removal, and (C) main peak analysis by SE-HPLC for product quality. L, load. In case of missing bars, the measured values were below the detection limit of the assay or too small for visualization. HCP, host cell proteins; SE-HPLC, size exclusion high performance liquid chromatography.

uses and then continuously increased up to 22% of the load value.

Regarding the DNA content, the treatment with buffer resulted in noticeable increase beginning after three filter uses and reaching about 40% of the starting level after four uses (Figure 2B). In contrast, DNA levels remained undetectable throughout the whole series using acidic regeneration, while alkaline regeneration caused slight increases in DNA levels after seven uses, finally reaching a level of 23% at tenfold use. During alkaline and acidic regeneration, the product quality of the main peak monomer was well preserved yielding values $\geq$ 98%, whereas the values for the buffer reference declined to the range of 96% (Figure 2C).

Overall, these unexpected results indicated that a high clearance capacity of the PDD1 filter was preserved during regeneration and multiple use. Both, alkaline and acidic regeneration maintained satisfactory HCP and DNA removal across multiple cycles. While acidic treatment provided complete removal of DNA and only slightly elevated but stable HCP values up to 10 uses, alkaline treatment yielded almost complete HCP and DNA removal up to seven uses and then caused minor increase of both impurities pointing to still high, but slowly decreasing clearance capacity with further uses. The excellent monomer quality obtained up to the last performed cycles, however, indicated that the filter properties were not affected. Thus, the cycle experiments revealed surprisingly effective HCP and DNA removal by filter regeneration. In case of acidic treatment, filter uses up to 10 times were justified, in case of alkaline regeneration at least six filter uses appeared feasible.

3.1.2 | Filter With Synthetic Fiber Matrix

The effects of regeneration were investigated for a second filter type by using the XOSP filter composed of polyacrylic fibers and silica under the same experimental conditions. Regarding HCP removal, acidic regeneration was very effective maintaining HCP levels at minimal values for up to 10 uses which indicated a potential for even further reuse (Figure 3A). The almost complete removal of HCP to levels lower than 100 ppm (see Figure 3D) observed in this study confirmed the recently reported improved impurity removal for HCP and aggregates when comparing the XOSP filter to standard filters (Nguyen et al. 2019). In contrast, alkaline regeneration and the reference buffer treatment achieved an overall reduction, but not to the level of acidic regeneration, and then let to continuous HCP increases with ongoing cycles. Interestingly, the alkaline pretreatment of the filter appeared to affect the retention of HCP as indicated by the elevated value after first use and further increases during the two following cycles.

For DNA clearance, the acidic regeneration again provided hardly detectable values up to ten filter uses, while a small and constant level of residual DNA was observed for alkaline treatment starting with the second filter use (Figure 3B). This phenomenon suggested that full DNA binding capacity of this filter matrix was preserved during acidic treatment, while alkaline regeneration led to a partial loss of the DNA binding capacity, presumably through chemical modification that became effective after the first regeneration cycle. Surprisingly, even for the buffer reference efficient DNA retention up to five reuses could be observed, indicating the strong DNA

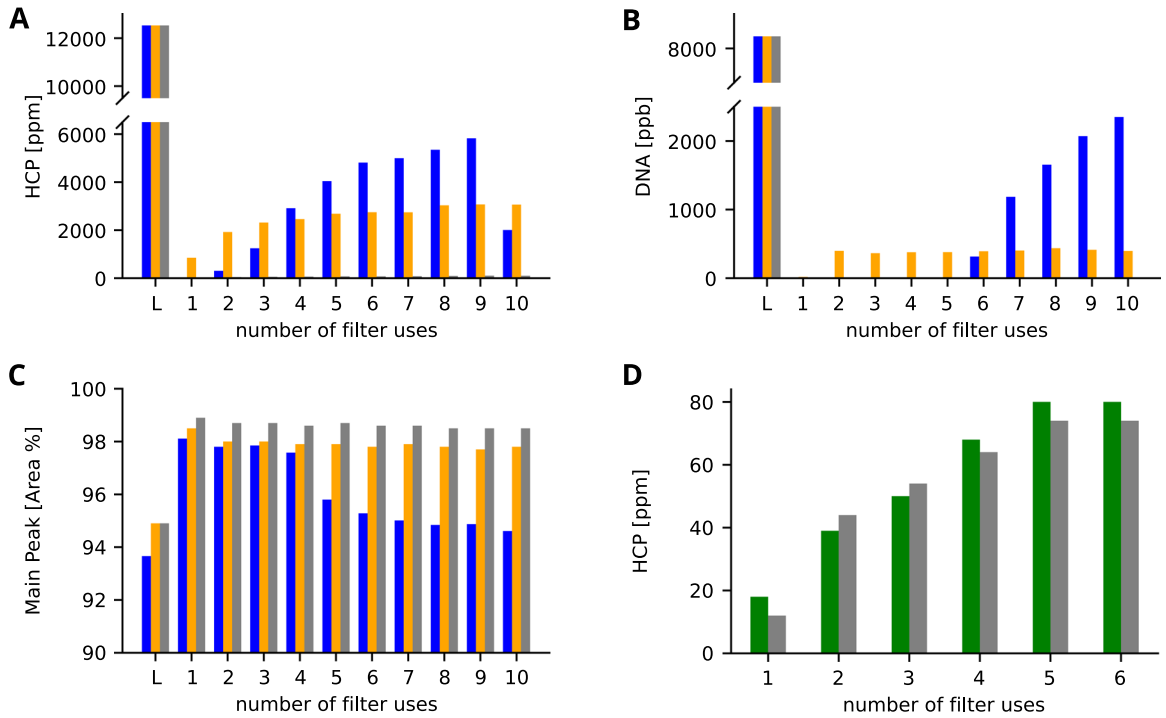

FIGURE 3 | Comparison of alkaline and acidic regeneration for the reuse of the synthetic depth filter XOSP (blue, equilibration buffer 150 mM acetic acid/tris pH 5.5; yellow, alkaline solution 1 M sodium hydroxide; gray, acidic solution 500 mM phosphoric acid) displaying (A) HCP removal, (B) DNA removal, and (C) main peak analysis by SE-HPLC for product quality. (D) displays HCP removal for acidic filter regeneration performed with 500 mM phosphoric acid (gray) or 167 mM acetic acid, 300 mM phosphoric acid (green). L, load. In case of missing bars, the measured values were below the detection limit of the assay or too small for visualization. HCP, host cell proteins; SE-HPLC, size exclusion high performance liquid chromatography.

removal capacity of this filter type. Again, the product quality was maintained at high levels over all reuses tested for acidic and alkaline regeneration, while it declined using the buffer reference (Figure 3C).

Thus, the results obtained for the synthetic XOSP filter apparently supported acidic regeneration for maintaining efficient clearance capacity during filter reuse. In contrast, HCP and DNA removals appeared somewhat affected with alkaline treatment indicating that sodium hydroxide was less compatible with this synthetic filter matrix. In addition to possible modifications of respective binding sites, this may also be related to the reported sensitivity of silica to alkaline treatment (Claessens et al. 1996) but was not further investigated in the scope of present study. In this regard, it is interesting that increasing pH values have been shown to increase the extraction of silicon from the XOSP filter matrix (Nguyen et al. 2019).

Taken together, this comparative study demonstrated that both types of filters could be efficiently regenerated for multiple use. The excellent clearance capacity retained with phosphoric acid clearly recommended acidic regeneration for the synthetic XOSP filter, while the cellulose-based PDD1 filter showed satisfactory performance with acidic and alkaline regeneration. However, the number of filter reuses appeared more limited for alkaline treatment than for acidic regeneration, where even more than 10 reuses seemed possible for both filters. The overall superior outcome of acidic regeneration induced the evaluation of a second regeneration solution which was milder and more common in laboratory practice. The comparison of 500 mM phosphoric acid with 167 mM acetic acid, 300 mM phosphoric acid as displayed in Figure 3D for the clearance of HCP during multiple filter reuse provided similar results indicating that the effectiveness of regeneration was identical. Therefore, the milder acidic solution was chosen in a further set of regeneration experiments.

3.2 | Acidic Regeneration Compared for Three Filter Types

To further confirm the reusability of depth filters by acidic regeneration, experiments using the milder acidic regeneration solution (167 mM acetic acid, 300 mM phosphoric acid) were extended to three filter types. The results obtained for XOSP and PDD1 regarding HCP, DNA, and product quality (Figures 4A–C and 5A–C) showed again efficient impurity removal, which was well comparable to the first reuse series and underlined the excellent regeneration properties of the mild acidic solution. Interestingly, the binding capacity for the impurities was retained by the use the equilibration solution, especially during the first two cycles, but thereafter the clearance performance declined compared to acidic treatment. The third filter VR02 was composed of cellulose fibers modified with charged surface groups. This filter appeared to have a generally weaker clearance capacity, since it left considerable higher levels of residual HCP (about 25%) and DNA with single use (Figure 6A,B) than the other two filters tested. However, acidic filter regeneration was effective because the initially achieved levels of HCP and DNA remained largely constant during filter reuse, whereas the buffer

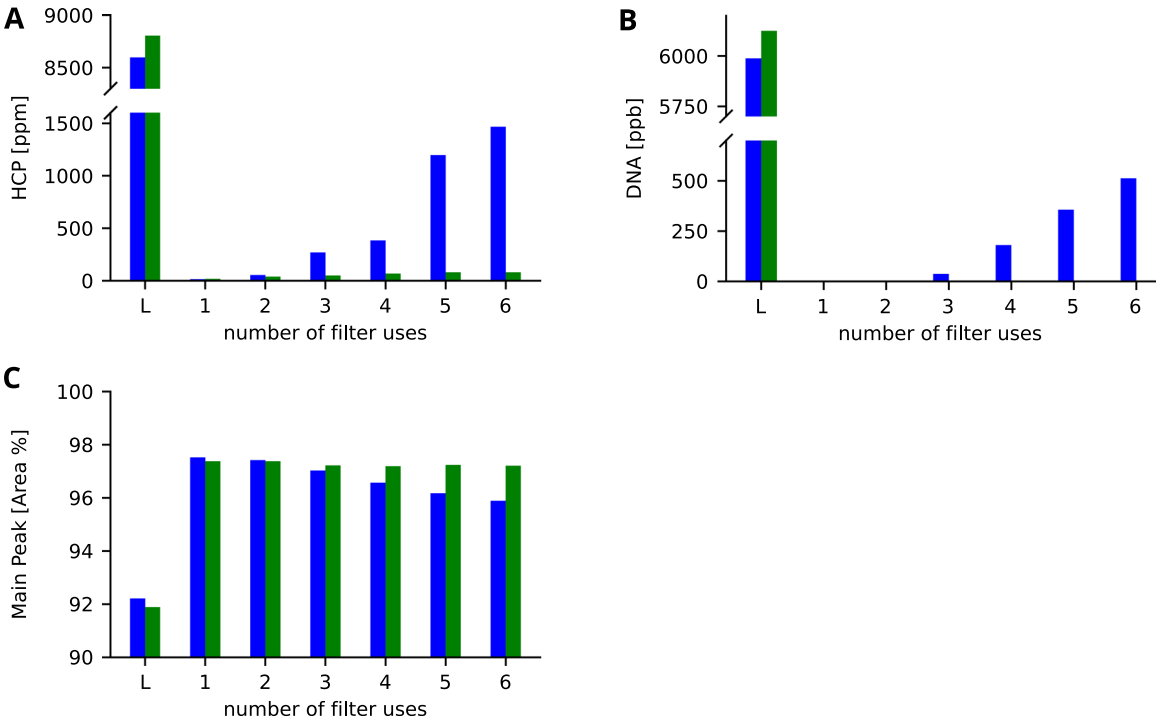

FIGURE 4 | Acidic regeneration for the reuse of the synthetic depth filter XOSP (blue, equilibration buffer 150 mM acetic acid/tris pH 5.5; green, 167 mM acetic acid, 300 mM phosphoric acid) displaying (A) HCP removal, (B) DNA removal, and (C) main peak analysis by SE-HPLC for product quality. L, load. In case of missing bars, the measured values were below the detection limit of the assay or too small for visualization. HCP, host cell proteins; SE-HPLC, size exclusion high performance liquid chromatography.

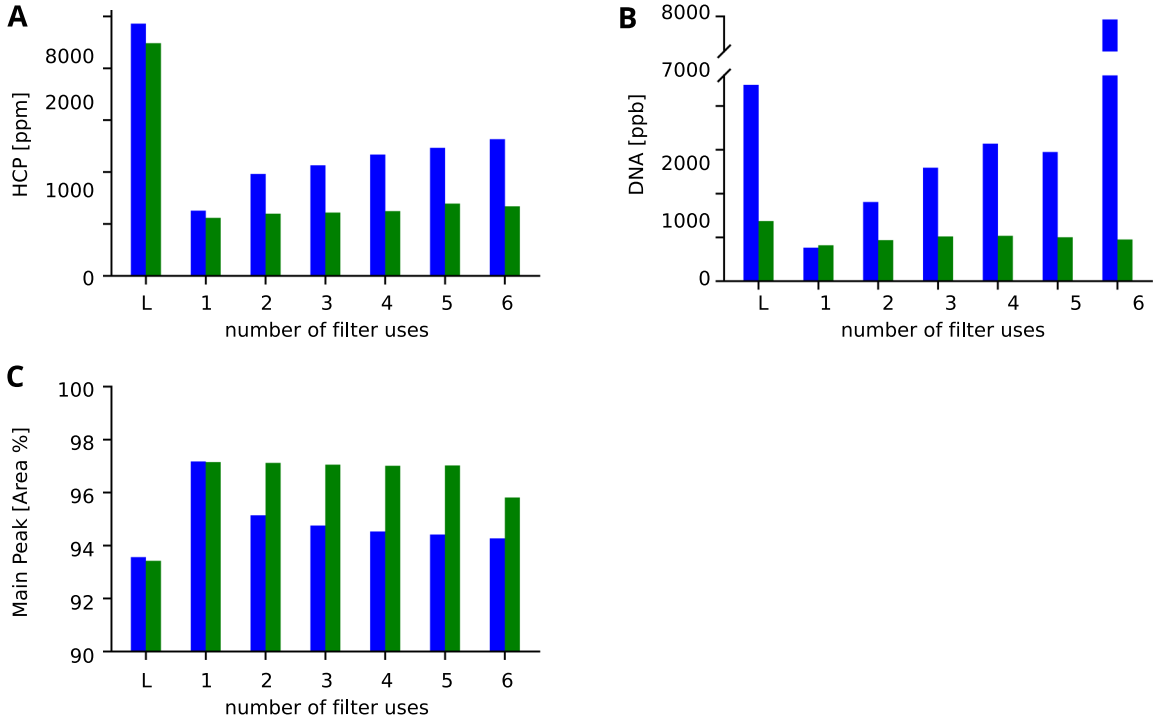

FIGURE 5 | Acidic regeneration for the reuse of the cellulose-based depth filter PDD1 (blue, equilibration buffer 150 mM acetic acid/tris pH 5.5; green, 167 mM acetic acid, 300 mM phosphoric acid) displaying (A) HCP removal, (B) DNA removal, and (C) main peak analysis by SE-HPLC for product quality. L, load. In case of missing bars, the measured values were below the detection limit of the assay or too small for visualization. HCP, host cell proteins; SE-HPLC, size exclusion high performance liquid chromatography.

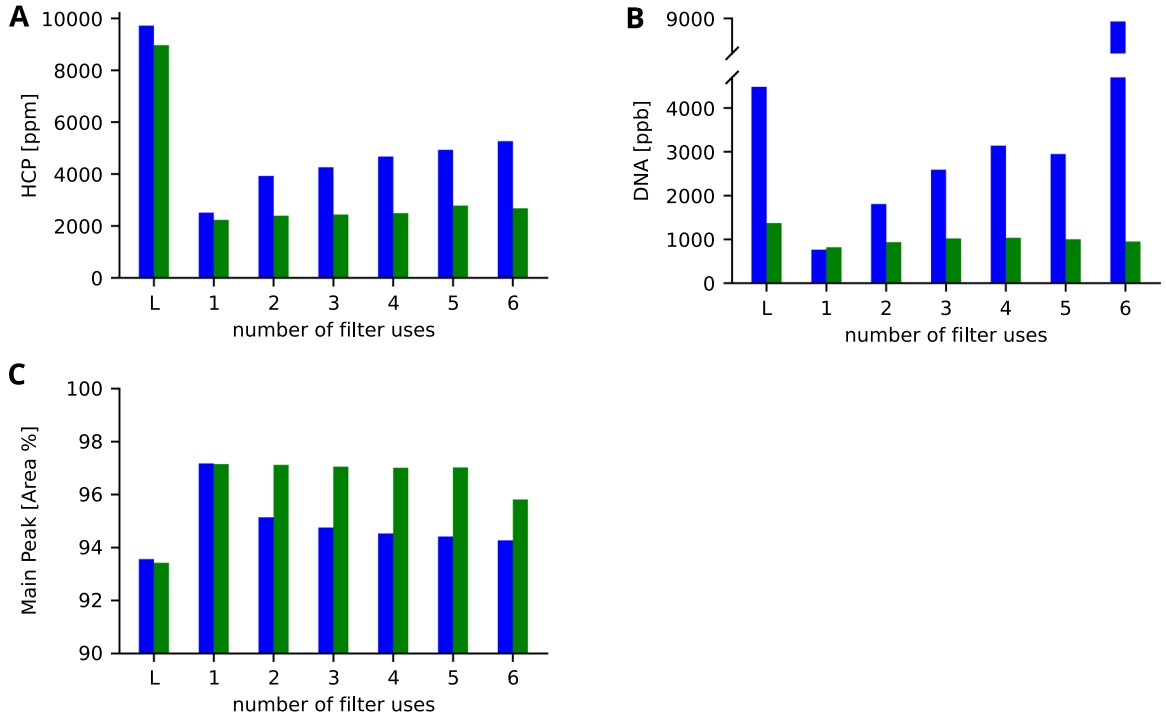

FIGURE 6 | Acidic regeneration for the reuse of the cellulose-based depth filter VR02 with charged surface groups (blue, equilibration buffer 150 mM acetic acid/tris pH 5.5; green, 167 mM acetic acid, 300 mM phosphoric acid) displaying (A) HCP removal, (B) DNA removal (six uses with an outlier for equilibration buffer), and (C) main peak analysis by SE-HPLC for product quality. HCP, host cell proteins; L, load; SE-HPLC, size exclusion high performance liquid chromatography.

reference showed a noticeable increase for both impurities. In addition, there was no evidence that acidic regeneration and reuse of the filter affected the high quality of the monomer product, which was in the same range as for the two other filters (Figure 6C). The consistent outcome seen for all three filter types supported the notion that acidic regeneration generally enables the reuse of depth filters and may be considered as a generic filter characteristic.

The excellent results in terms of monomer content and remaining clearance capacity for HCP and DNA indicated good stability of the filter matrix upon multiple reuses as well as adequate resistance to recurring regeneration conditions.

Obviously, the possibility of multiple depth filter reuses reduces the burden on the supply chain. However, the reuse of filters also offers operational advantages during downstream processing, as the availability of filter area becomes more flexible. In this regard, filter regeneration enables an increase in usable filter area and capacity, thus avoiding high loads and the risks of filter overloading, blocking, and impurity breakthrough, which greatly supports the implementation of robust procedures. Although tradeoffs are needed to evaluate specific scenarios, the flexibility gained and the savings in the CO_2 footprint offer the potential to more than compensate for the increase in time required for the regeneration process. This assumption is supported by the fact that the present results are based on maximal regeneration times and solution volumes that may be optimized for shortened incubation steps and reduced solution consumption. Further adjustments in this regard would be particularly relevant for continuous manufacturing approaches.

3.3 | Sustainability Aspects

The results of this study clearly demonstrate that depth filter regeneration enables multiple use of filters instead of the standard single use application. In addition to the obvious benefits of saving raw materials and plastic waste, multiple use may reduce the environmental impact of the purification process. To give a more detailed estimation of the savings potential, the procedure using ten different filter units in single use was opposed to the tenfold use or reuse of a single filter unit as illustrated in Figure 1 for a 2000 L depth filtration setup corresponding to about 3300 g protein, which represents a common scale in production processes. Based on the assumptions for the consumption of materials and buffers (Table 1), the assessed saving potential was calculated as carbon dioxide equivalents (Figure 7) showing approximately fourfold lower carbon dioxide emissions for each of the three presented filter reuse protocols than for the single use procedure. For acidic regeneration with 167 mM acetic acid, 300 mM phosphoric acid, the saving potential accounted for 522 kg CO_2 equivalents per 2k batch. The more than fourfold difference resulted from the fact, that the filter made up the main part of the calculation, while the contribution of the additionally used regeneration solutions was marginal. Although there is some uncertainty due to the estimation of the filter value, Figure 7 reveals that even with a lower filter portion, the remaining difference between single use and reuse would still be significant. Furthermore, the presented results (Figures 2 and 3) suggest that the filters may be used even more than 10 times, thus offering additional options for savings.

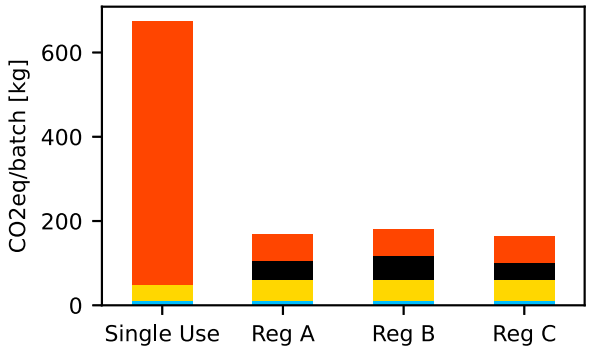

FIGURE 7 | Calculation of the carbon footprint (kg CO_2 equivalents) of a 2000-L (2k) filtration setup comparing the procedure with 10 depth filter units in single use to that with tenfold use of a single filter unit applying the regeneration solutions Reg A (500 mM phosphoric acid), Reg B (1 M sodium hydroxide), and Reg C (167 mM acetic acid, 300 mM phosphoric acid). The bars are composed of water (blue), buffer chemicals (yellow), regeneration solution (black), and filter (orange).

Thus, the present study demonstrates that multiple use of depth filters can contribute to reduce carbon dioxide emissions and improve overall sustainability of downstream processing at the manufacturing scale. These findings challenge the current trend toward increasing applications of single use technologies (Lalor et al. 2019) and point toward elongating the life cycle of materials, which are typically used as disposables (Whitford et al. 2022). The savings effect will also become more important from an economic perspective as soon as the carbon dioxide price will continue to rise.

4 | Conclusion

The present study on the regeneration capability of depth filters reveals the unexpected finding that the filters can be reused without loss of secondary clearance performance, as assessed by the removal of HCP and DNA impurities form affinity-captured mAb preparations. To achieve multiple use, acidic as well as alkaline regeneration may be applicable depending on the filter composition. However, acidic regeneration appears more effective and consistent, as suggested by the evaluation of three filter types where at least 10 reuses appeared possible for the synthetic filter. In addition to unburdening the supply chain, the reuse of filters improves the availability of filter area during processing, facilitates implementation of robust procedures and supports the sustainability of biopharmaceutical drug production by reducing waste and carbon dioxide emissions. In view of the advantages offered by the reuse of depth filter in secondary clarification, it would be worth to investigate multiple use of filters in primary clarification as well, although the challenge for effective regeneration may be greater due to a higher content of cell debris and impurities. However, based on the surprising findings of this study, it is not unlikely that regeneration will work even under more stringent conditions or that the procedures can be adapted accordingly. Moreover, the findings of the present study are also applicable to the processing of biotherapeutic formats other than mAbs.

Author Contributions

Bernhard Spensberger: conceptualization, methodology, investigation, formal analysis, visualization, final proofreading. **Marc Pompiati:** visualization, investigation, final proofreading. **Christoph Feistl:** visualization, investigation, final proofreading. **Thorsten Lemm:** visualization, funding acquisition, final proofreading. **Ferdinand Stückler:** conceptualization, methodology, formal analysis, visualization, final proofreading. **Roberto Falkenstein:** conceptualization, methodology, visualization, supervision, final proofreading.

Acknowledgments

The authors thank Heidemarie Peuker (BISs - Biomedical Investigation Services) for diligent preparation of the manuscript and fruitful discussions. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Data Availability Statement

The authors have nothing to report.

References

Berggrund, A., I. Drevin, K.-G. Knuuttila, J. Wardhammar, and B.-L. Johansson. 1994. "Chemical and Chromatographic Characterization of a New Bioprocess Medium for Hydrophobic Interaction Chromatogrpahy: Butyl Sepharose 4 Fastflow." *Process Biochemistry* 29: 455–463.

Budzinski, K., D. Constable, D. D'Aquila, et al. 2022. "Streamlined Life Cycle Assessment of Single Use Technologies in Biopharmaceutical Manufacture." *New Biotechnology* 68: 28–36. https://doi.org/10.1016/j.nbt.2022.01.002.

Burgoyne, R. F., M. C. Priest, K. L. Roche, and G. Vella. 1993. "Systematic Development and Validation of Sanitization Protocols for a Chromatographic System Designed for Biotherapeutics Purification." *Journal of Pharmaceutical and Biomedical Analysis* 11, no. 11–12: 1317–1325. https://doi.org/10.1016/0731-7085(93)80118-k.

Chandler, M. A., and A. L. Zydney. 2005. "Clarification of Yeast Cell Suspensions by Depth Filtration." *Biotechnology Progress* 21, no. 5: 1552–1557. https://doi.org/10.1021/bp0500821.

Charlton, H. R., J. M. Relton, and N. K. H. Slater. 1999. "Characterization of a Generic Monoclonal Antibody Harvesting System for Adsorption of DNA by Depth Filters and Various Membranes." *Bioseparation* 8, no. 6: 281–291. https://doi.org/10.1023/a:1008142709419.

Claessens, H. A., M. A. van Straten, and J. J. Kirkland. 1996. "Effect of Buffers on Silica-Based Column Stability in Reversed-Phase High-Performance Liquid Chromatography." *Journal of Chromatography A* 728: 259–270.

EcoInvent version 3.9.1. Published October 29, 2023. https://support.ecoinvent.org/ecoinvent-version-3.9.1.

Ghose, S., T. M. McNerney, and B. Hubbard. 2004. "Preparative Protein Purification on Underivatized Silica." *Biotechnology and Bioengineering* 87, no. 3: 413–423. https://doi.org/10.1002/bit.20125.

Grönberg, A., M. Eriksson, M. Ersoy, and H. J. Johansson. 2011. "A Tool for Increasing the Lifetime of Chromatography Resins." *mAbs* 3, no. 2: 192–202. https://doi.org/10.4161/mabs.3.2.14874.

Hogwood, C. E. M., A. S. Tait, N. Koloteva-Levine, D. G. Bracewell, and C. M. Smales. 2013. "The Dynamics of the Cho Host Cell Protein Profile During Clarification and Protein a Capture in a Platform Antibody Purification Process." *Biotechnology and Bioengineering* 110, no. 1: 240–251. https://doi.org/10.1002/bit.24607.

Holstein, M., D. Jang, C. Urrea, et al. 2021. "Control of Leached Beta-Glucan Levels From Depth Filters by an Improved Depth Filtration

Flush Strategy." *Biotechnology Progress* 37, no. 1: e3086. https://doi.org/10.1002/btpr.3086.

Iskra, T., G. R. Bolton, J. L. Coffman, and R. Godavarti. 2013. "The Effect of Protein A Cycle Number on the Performance and Lifetime of an Anion Exchange Polishing Step." *Biotechnology and Bioengineering* 110, no. 4: 1142–1152. https://doi.org/10.1002/bit.24781.

Kandula, S., S. Babu, M. Jin, and A. A. Shukla. 2009. "Design of a Filter Train for Precipitate Removal in Monoclonal Antibody Downstream Processing." *Biotechnology and Applied Biochemistry* 54, no. 3: 149–155. https://doi.org/10.1042/BA20090181.

Khanal, O., N. Singh, S. J. Traylor, et al. 2018. "Contributions of Depth Filter Components to Protein Adsorption in Bioprocessing." *Biotechnology and Bioengineering* 115, no. 8: 1938–1948. https://doi.org/10.1002/bit.26707.

Khanal, O., X. Xu, N. Singh, et al. 2019. "DNA Retention on Depth Filters." *Journal of Membrane Science* 570–571: 464–471. https://doi.org/10.1016/j.memsci.2018.10.058.

Koehnlein, W., A. Holzgreve, K. Schwendner, R. Skudas, and F. Schelter. 2023. "Purification of Hydrophobic Complex Antibody Formats Using a Moderately Hydrophobic Mixed Mode Cation Exchange Resin." *Journal of Chromatography A* 1687: 463696. https://doi.org/10.1016/j.chroma.2022.463696.

Koehnlein, W., E. Kastenmueller, T. Meier, T. Treu, and R. Falkenstein. 2024. "The Beneficial Impact of Kosmotropic Salts on the Resolution and Selectivity of Protein A Chromatography." *Journal of Chromatography A* 1715: 464585. https://doi.org/10.1016/j.chroma.2023.464585.

Lalor, F., J. Fitzpatrick, C. Sage, and E. Byrne. 2019. "Sustainability in the Biopharmaceutical Industry: Seeking a Holistic Perspective." *Biotechnology Advances* 37, no. 5: 698–707. https://doi.org/10.1016/j.biotechadv.2019.03.015.

Liu, H. F., J. Ma, C. Winter, and R. Bayer. 2010. "Recovery and Purification Process Development for Monoclonal Antibody Production." *mAbs* 2, no. 5: 480–499. https://doi.org/10.4161/mabs.2.5.12645.

Nejatishahidein, N., E. E. Borujeni, D. J. Roush, and A. L. Zydney. 2020. "Effectiveness of Host Cell Protein Removal Using Depth Filtration With a Filter Containing Diatomaceous Earth." *Biotechnology Progress* 36, no. 6: e3028. https://doi.org/10.1002/btpr.3028.

Nguyen, H. C., A. L. Langland, J. P. Amara, M. Dullen, D. S. Kahn, and J. A. Costanzo. 2019. "Improved HCP Reduction Using a New, All-Synthetic Depth Filtration Media Within an Antibody Purification Process." *Biotechnology Journal* 14, no. 1: e1700771. https://doi.org/10.1002/biot.201700771.

Parau, M., T. F. Johnson, J. Pullen, and D. G. Bracewell. 2022. "Analysis of Fouling and Breakthrough of Process Related Impurities During Depth Filtration Using Confocal Microscopy." *Biotechnology Progress* 38, no. 2: e3233. https://doi.org/10.1002/btpr.3233.

Schreffler, J., M. Bailley, T. Klimek, et al. 2015. "Characterization of Postcapture Impurity Removal Across an Adsorptive Depth Filter." *BioProcess International* 13: 36–45.

Shukla, A. A., B. Hubbard, T. Tressel, S. Guhan, and D. Low. 2007. "Downstream Processing of Monoclonal Antibodies--Application of Platform Approaches." *Journal of Chromatography B* 848, no. 1: 28–39. https://doi.org/10.1016/j.jchromb.2006.09.026.

Singh, N., A. Arunkumar, S. Chollangi, Z. G. Tan, M. Borys, and Z. J. Li. 2016. "Clarification Technologies for Monoclonal Antibody Manufacturing Processes: Current State and Future Perspectives." *Biotechnology and Bioengineering* 113, no. 4: 698–716. https://doi.org/10.1002/bit.25810.

Singh, N., K. Pizzelli, J. K. Romero, et al. 2013. "Clarification of Recombinant Proteins From High Cell Density Mammalian Cell Culture Systems Using New Improved Depth Filters." *Biotechnology and Bioengineering* 110, no. 7: 1964–1972. https://doi.org/10.1002/bit.24848.

Whitford, B., D. Jones, and S. Kinnane. 2022. "Biomanufacturing Design: Reducing the Environmental Burden." *Current Opinion in Biotechnology* 76: 102717. https://doi.org/10.1016/j.copbio.2022.102717.

Yigzaw, Y., R. Piper, M. Tran, and A. A. Shukla. 2006. "Exploitation of the Adsorptive Properties of Depth Filters for Host Cell Protein Removal During Monoclonal Antibody Purification." *Biotechnology Progress* 22, no. 1: 288–296. https://doi.org/10.1021/bp050274w.

Yu, D., M. Mayani, Y. Song, Z. Xing, S. Ghose, and Z. J. Li. 2019. "Control of Antibody High and Low Molecular Weight Species by Depth Filtration-Based Cell Culture Harvesting." *Biotechnology and Bioengineering* 116, no. 10: 2610–2620. https://doi.org/10.1002/bit.27081.

Supporting Information

Additional supporting information can be found online in the Supporting Information section.