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# Optimization of a Cell Culture Clarification Platform Process With the Aim of Boosting the Recovery of Bispecific Antibodies

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## Context and Introduction

The development of a process for the manufacturing of a biological drug is a long and complex exercise. With the increasing numbers of biological drugs in clinical evaluation, many companies have implemented platform approaches to reduce timelines to start clinical trials.

| | Centrifugation | Tangential-Flow Filtration | Depth Filtration |
|---|---|---|---|
| Investment | High | Intermediate | Low |
| Free of particles | No | Yes | Yes |
| Maximum culture volume | 6,000 L | 2,000 L | "Unlimited" |
| Suitable for continuous processing? | Yes | Yes (ATF) | No |
| Scale-down model available? | No | Yes | Yes |

Table 1: Comparison of single-use harvest clarification technologies [1]

Considerable research efforts were made to improve upstream productivity by achieving higher cell densities and a longer culture duration. As a consequence, this has increased the burden on the clarification and purification steps to efficiently remove increased levels of biomass and impurities, while achieving maximum of product recovery.

Clarification is a critical step that removes early contaminants in the process. This step is mainly focused on the clearance of large and medium particles like whole cells and cell debris.

Three main methods are available to perform clarification: centrifugation, tangential flow filtration (TFF) or depth filtration. Each technology has its advantages, and a plethora of combinations of these technologies exists to improve product recovery and thus the clarification process. Additionally, new technologies are now available, using acoustic separation, flocculation, or the combination of depth filtration and TFF principles (TFDF™, Repligen).

Ichnos' clarification process focuses on depth filtration technology only, as the volume to clarify remains low.

A typical depth filtration allows impurity removal based on molecular interactions (electrostatic, hydrophobic and hydrogen bonding, adsorption) in addition to particle entrapment linked to hydrodynamic interactions (size-based sieving, interception, and cake-filtration).

The adsorption effect is responsible for binding DNA and Host Cell Proteins (HCP) but can also unfortunately bind the product of interest and lead to a recovery loss. Depending on the isoelectric point (pI) of the product, the antibody can be bound to the membrane. Based on specific residues present in the molecule, hydrophobic interactions can also happen between the filter and the product.

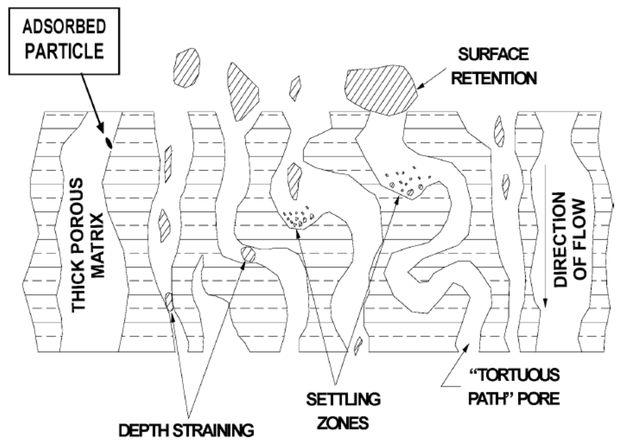


Figure 1: Mechanisms of particle retention in depth filtration [2]

During clarification, product loss can be experienced because of the molecule binding characteristics. Herein, alternatives to maximize the product recovery are identified.

[1] Schmidt et al. (2017), "Single-use Depth Filters: application in clarifying industrial cell cultures", BioProcess International

[2] Roush D. et al. (2008), "Advances in primary recovery: centrifugation and membrane technology", Biotechnology Progress

## Ichnos' Clarification Process Overview

At Ichnos Sciences, clarification is carried out with two different single-use synthetic depth filters used in series. This allows the removal of a wide range of particles, from 15 µm to less than 0.1 µm. The primary filter clears cells and cell debris while the second filter removes smaller impurities like HCP and DNA.

Platform clarification is composed of 5 steps: a water for injection (WFI) flush, a 1 X phosphate buffer saline (PBS) 140 mM flush, a dead volume (DV) discard step, the clarification and a 1 X PBS 140 mM recovery flush.

| Step ID | Step | Scale up based on LMH | Minimum volume | Surface of membrane | Time | Flow rate |
|---|---|---|---|---|---|---|
| | | L/m²/hour | L | m² | min | mL/min |
| #1 | WFI flush | 300 | 2.081 | 0.0270 | 15.4 | 13 |
| #2 | PBS flush | 300 | 1.720 | 0.0270 | 12.7 | 135 |
| #3 | Dead volume discard | 100 | 0.541 | 0.0270 | 12 | 45 |
| | | | 0.347 | 0.0140 | 15 | 23 |
| | | | 0.888 | 0.0410 | 13 | 68 |
| #4 | Clarification | 70 | 2.500 | 0.0270 | 79 | 32 |
| #5 | PBS flush | 70 | 1.776 | 0.0270 | 56 | 32 |

Table 2: Overview of the Ichnos clarification platform steps

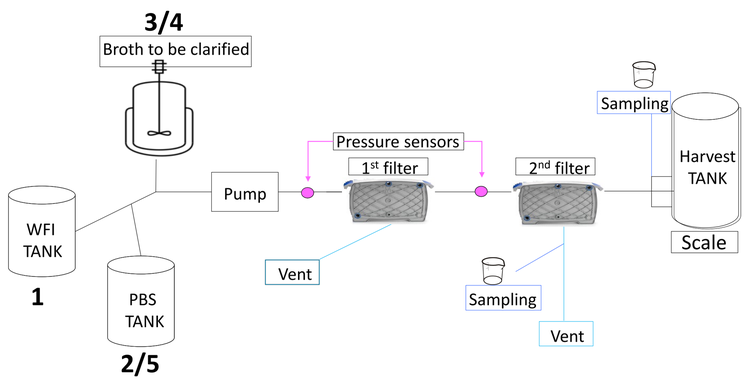


Figure 2: Detailed Ichnos clarification platform process flow

The WFI flush removes all leachables and extractables remaining in the filters from the manufacturing process. The first PBS flush equilibrates the filter media to a suitable pH. The dead volume discard step limits the dilution of the product by removing the filter void volume. Finally, the second PBS flush aims to recover a maximum of product that is remaining in the filters. At least one DV of PBS needs to be flushed to recover the harvest volume remaining in the filters. Another DV is added to unbind the remaining product bound to the filter media.

Bench scale clarification platform filters were mainly used. Small scale filters were also tested for screening purpose.

The clarification runs were conducted based on the platform limits, meaning a maximum of pressure of 2.4 bars at bench scale (filter limit – supplier recommendation) and a turbidity of 20 NTU post clarification for both scales.

## Product Recovery Yields With Ichnos' Antibodies Formats

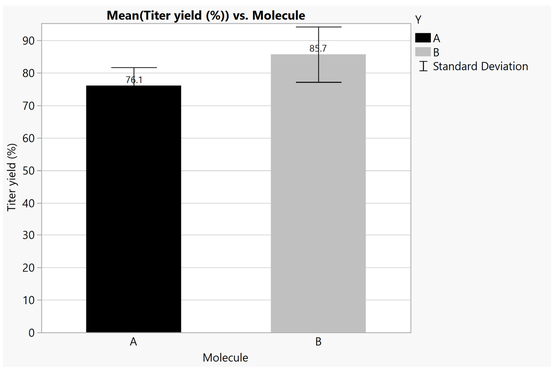


Figure 3: Product recovery yields for two Ichnos molecules
*Error bar is 1 standard deviation from the mean. N = 13 for molecule A and N = 7 for molecule B.*

Given the increase in biomass accumulation and in productivity, the clarification platform was optimized using synthetic filters with a model molecule. With the platform described above, successful clarifications using a broth with turbidity above 5000 NTU and high titers were achieved with yields above 90%.

However, for molecules A and B an average yield of 76% and 86% was achieved. Hypotheses were drafted to explain this difference.

**Molecule A = bi-specific, low titer, low turbidity**

**Molecule B = IgG-like, high titer, high turbidity**

→ **Molecule structure?**
**Saturation phenomenon?**
**Binding competition impurities vs. product?**

Figure 4: Overview of the list of hypotheses for product recovery differences

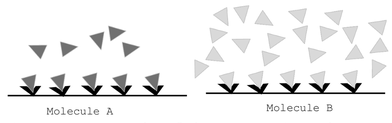


Figure 5: Example of the saturation phenomenon

The first hypothesis is linked to the fact that Molecule A is a BEAT® bispecific antibody[3] whereas molecule B is a classical antibody. Molecule A is more charged and hydrophobic due to its higher mass and will more strongly interact with the membrane.

A product-specific saturation phenomenon can be listed as a second hypothesis. Indeed, the concentration of molecule B is higher in the initial broth. Assuming a defined number of binding sites on the membrane, the yield will be impacted (Figure 5).

The last hypothesis is about non-specific binding competition between the product and the impurities. Molecule B broth is higher in turbidity and contains more HCPs, which may lead to binding competition to the membrane, increasing titer recovery.

[3] BEAT®, Bispecific Engagement by Antibodies based on the T-cell receptor

## Evaluation of Arginine-HCl as an Alternative Flushing Buffer

Arginine-HCl buffer was identified as a potential buffer to remove both charged and hydrophobic interactions[4], [5].

For molecule A, adding arginine-HCl increased recovery by 14% when compared to the current platform process. A significant difference can be observed with the ANOVA test, with a mean of 86% recovery post arginine-HCl versus 72% post PBS. The results indicate that the new buffer weakens the interactions with the matrix on molecule A.

Molecule B cell culture process was optimized when compared to historical data (Figure 3) and the product titer was increased by a factor of 10. When flushing with arginine-HCl using the high titer broth, an increase of 4% in recovery was observed (Figure 6). In comparison, when arginine-HCl was tested on the low titer broth before process optimization, recovery increased by 14% (Figure 7, blue dot at low titer). This suggests that the saturation hypothesis is the most probable reason for product recovery issues independent of the molecule format.

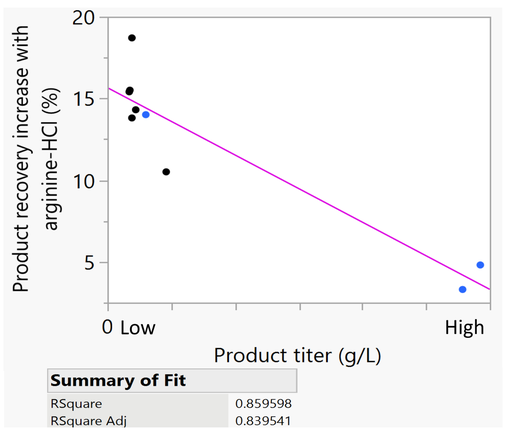


Figure 7: Linear correlation between product recovery with arginine-HCl and initial product titer
***Black dots** = molecule A, **Blue dots** = molecule B*

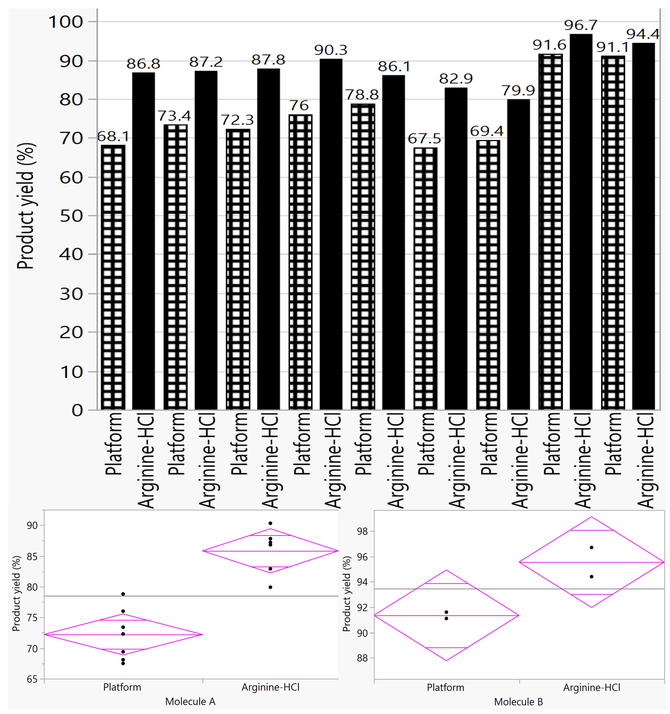


Figure 6: Increase of product recovery with arginine-HCl flush

Maximum recovery was achieved with low initial titers independent of the molecule. An R-square adjusted of 0.84 shows the degree of correlation.

Arginine-HCl can then be used as an alternative flush to boost product recovery in low initial titer projects, independent of the antibody format. The effect of this flush was also evaluated in the next downstream steps without any detectable issue.

In terms of investment, the process cost only increased slightly with this new buffer (+2%).

[4] Arakawa T. et al. (2007), "The effects of arginine on binding and elution in hydrophobic interaction and ion-exchange chromatography", Protein Expression and Purification

[5] Ejima D. et al. (2005), "Arginine as an effective additive in gel permeation chromatography", Journal of Chromatography A

## Evaluation of Cellulosic-based HC Filters as an Alternative

As shown with the platform process (Figure 3), molecule A resulted in lower recoveries than molecule B.

Another type of filter (HC filters, Merck) was evaluated in 4 different experiments.

The same platform process was applied, except that the WFI and PBS initial steps were increased to 100 L/m² for WFI flush and 50 L/m² for the PBS equilibration.

The increase in product recovery was 29% on average, with more than 95% of the antibody collected in the harvest container. This showed that no product interaction occurred with the cellulosic-based filters (Figure 8).

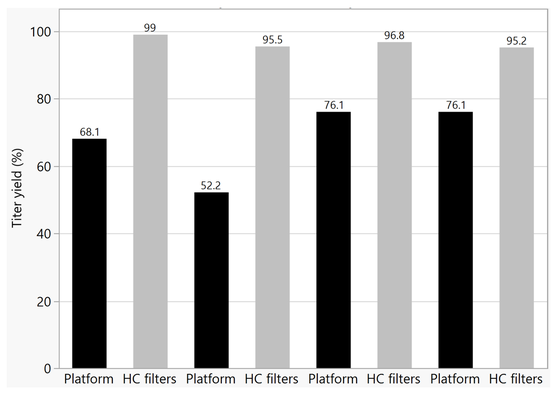


Figure 8: Increase of product recovery with HC filters

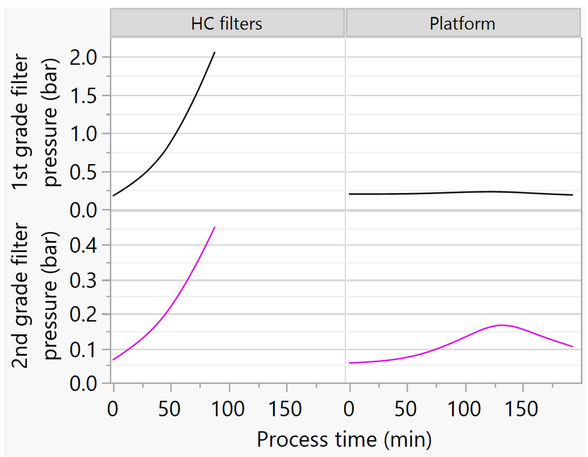


Figure 9: Pressure monitoring between HC and platform processes

The filter's capacity, which was limited by the pressure, was identified as the main issue. When the platform was designed using a model molecule, these filters were excluded as the filterability was too low to be economically viable.

With the molecule A, the first-grade filter pressure increased up to 2 bars at a throughput of 100 L/m². The second filter followed the same pressure trend, demonstrating a clogging at the same throughput. The current platform process which uses synthetic filters, shows at least twice the capacity (Figure 9). When a safety margin surface factor of 1.5 is considered for the confirmation trial (trial 4), no pressure increase was detected.

Even if the consumable cost is increasing (more surface), the recovery gain is decreasing the cost of 1 gram of product at the end of the process, compared to the platform (-19 %).

## Conclusions

Screening of clarification process parameters allowed detection of two promising improvements for yield optimization of the current platform:

- ✓ Flushing with arginine-HCl instead of PBS increased the molecule A recovery by 14 %. However, arginine-HCl did not show a significant improvement in product recovery with molecule B in this study (Figure 6).
- ✓ The data (Figure 7) indicate that the recovery differences are not linked to the molecule format, but to the initial titer. It could be speculated that the higher the initial titer level in the broth, the lower the impact of arginine-HCl on product yield. This would be well-aligned with the assumption that the saturation effect of the binding sites of the filters becomes negligible with higher initial titers.
- ✓ Using HC filters allowed the recovery of more than 95 % of molecule A. Nevertheless, HC filters showed limitations in terms of pressure. A final throughput of 65 L/m² was determined as a target to avoid any pressure build-up.
- ✓ From a process cost perspective, HC filters would be the most cost-effective solution compared to arginine-HCl. Indeed, HC filters would decrease the cost to produce 1g of product (-19 %) due to the high product recovery.
- ✓ In contrast, due to the high price of the buffer, the gain in product yield with arginine-HCl results in a slight increase in cost (+2 %) to produce 1 g of product.