CELL HARVEST WITH CONTIBAC® SU FILTERS

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Introduction

Throughout the past two decades, single-use (SU) components have been on the rise in the production of biologics, since they reduce the risk of cross-contamination and render costly equipment sterilizations and validations unnecessary. The shift towards SU components has been successful for many production steps, yet some challenges remain. Particularly in cell separations, current SU technologies are not capable of handling the high batch volumes that are the norm in non-SU plants [1-2].

The technologies that are most widely used for SU cell harvest (for extracellular applications) are depth filtration, centrifugation, and cross-flow filtration [3]. Depth filters are straightforward in concept and operation, but have several draw-backs. The major issue is the large footprint and the number of required modules, which becomes problematic as batch volumes and cell densities increase. Moreover, depth filters cannot be regenerated and are more susceptible to turbidity breakthrough [4]. Lastly, depth filters are costly, which is why the BioPhorum Operations Group recommended the development of alternative filtration methods and devices [5]. Centrifugation is widely used in production plants of biologics with working volumes over 2'000 L. Converting the technology from stainless steel to SU, however, requires complex equipment and intricate SU components,

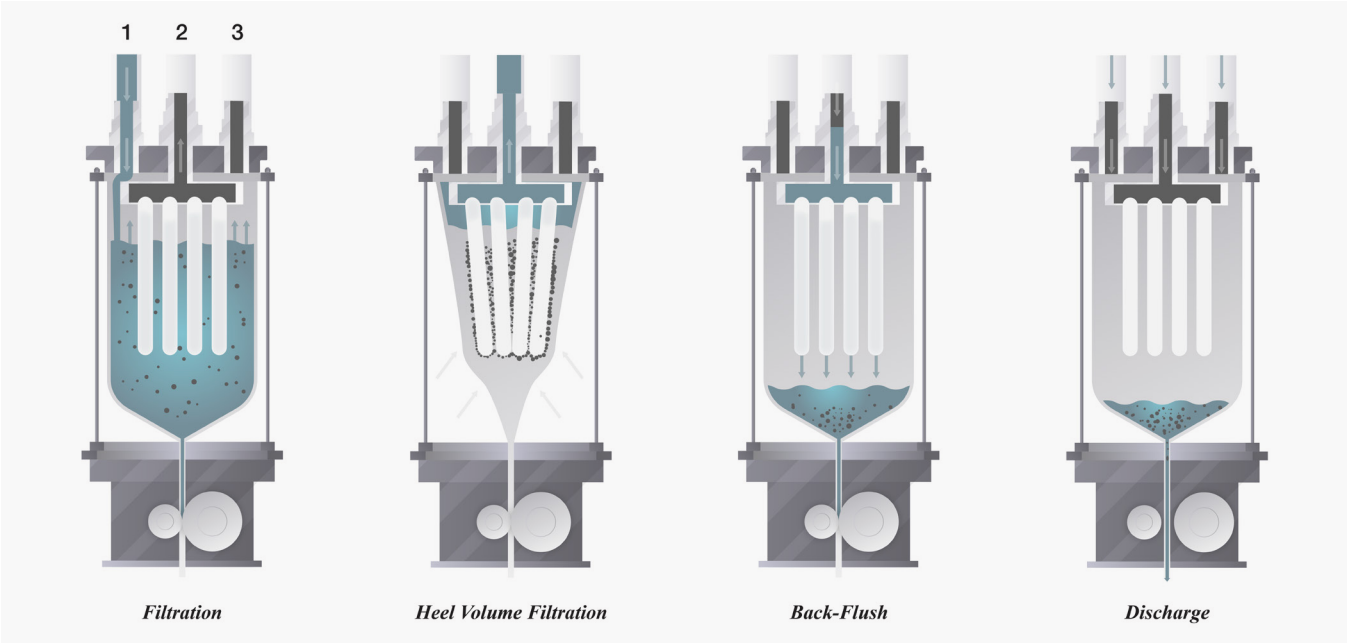

Figure 1: Filtration steps that comprise one complete cycle of the CONTIBAC SU® [3].

resulting in high investment and operating costs. Cross-flow filters have rather low filtration rates, while requiring a very high cross-flow velocity, which is energy inefficient and can harm shear-sensitive mammalian cells [6]. Also, the solids concentration in the feed tank increases throughout the process. Eventually, the solid concentration becomes too high to be pumped through the filter, and the remaining liquid product in the tank is lost.

As the industry is moving towards larger batches, higher cell densities, and continuous production, the aforementioned technologies are pushed to the limit, and there is a demand for innovative technologies. The CONTIBAC® SU of DrM, Dr. Mueller AG, introduced in this study, overcomes some of these limitations by using two novel concepts:

(1) Cake Filtration: The filter medium does not perform the actual filtration, but it acts as a support for the filter cake consisting of cells and filter aid.

Figure 2: 0.2L Nutsche that was used for the experiments in Figs. 5-8.

As a result, the filtration is much faster, and the filter medium can be regenerated by being back-flushed from the opposite side.

(2) Cyclic Operation: Since the filter medium can be regenerated, the filter can be operated in a cyclic manner that is illustrated in Figure 1 and explained in the following paragraph.

A cross-section of the filter is illustrated in Figure 1. The filter consists of multiple vertically aligned elements, each containing a filter medium around the circumference. These filter elements are connected with each other on the top, such that the filtrate from each element is collected in a horizontal register and can exit the filter through connector 2. The filter elements are completely encapsulated by a polyethylene bag, and there is an air-tight seal between the bag and the filter housing.

As shown in Figure 1, there are four steps in a complete filtration cycle. During the first step, the filter is filled through connector 1. Once filled (having allowed the air to escape through connector 3), the liquid pushes through the filter media into the vertical elements and exits the filter through connector 2. As the filtration is carried on a cake forms on each element. When the cake has grown to a degree that causes a significant drop in the flow rate, it is proceeded to the next step, the heel volume (HV) filtration. Air is pumped between the filter bag and the filter housing, squeezing all the remaining liquid out of the bag. The benefit of this step is that all the product is harvested, unlike in depth filters and cross-flow filters where the heel volume cannot be filtered, resulting in a yield reduction. After completing the HV filtration, the filter madia are back-flushed by pumping water for injection (WFI) or buffer through connector 2 (i.e. reversing the flow). As a result, the cake is removed from the filter elements and accumulates as a slurry on the bottom of the filter bag. This slurry is removed from the filter bag in the last step by opening the bottom pinch valve. The back-flush step completely regenerates the filter media for the next cycle, while the discharge step regenerates the whole filter bag for the next cycle.

The advantage of using the aforementioned cyclic filtration technique is that a smaller filter volume can be used to perform the same task. Unlike conventional filters, whose capacity is limited by the filter area, the capacity of the CONTIBAC® SU filter is only limited by the number of cycles, or respectively, the time the user has allotted for the filtration. A smaller filter also leads to a smaller footprint, a lower contact area (and hence less leachables and extractables), as well as lower investment and operating costs. Finally, the CONTIBAC® SU can be used for the quasi-continuous production of biologics that spans over several days or weeks, something that is rather challenging to achieve with conventional filters.

While these conceptual advantages of the CONTIBAC® SU have been discussed in another study [3], they have not yet been undermined by scientific evidence. Providing

quantitative arguments in favor of the CONTIBAC® SU was the purpose of this study.

Experimental Procedures

Suspension growing Chinese Hamster Ovary (CHO) DP-12 cells, producing an Immunoglobulin (IgG)-1 antibody against Interleukin-8 (clone #1934, ATCC CRL-12445, provided by Prof. Dr. T. Noll, Bielefeld University, Germany), were cultivated in a chemically defined medium. Three suspensions of this cell line were tested in this study, henceforth referred to as cultures 1, 2 and 3. Culture 1 was cultivated in a wave-mixed bioreactor with 5 L working volume in batch mode (Sartorius Stedim Biotech), culture 2 was cultivated in a 50 L stirred single-use bioreactor (Sartorius Stedim Biotech) in fed-batch mode, while culture 3 was cultivated in a wave-mixed bioreactor with 1 L working volume in continuous mode.

Before starting the filtration, the wet cell weight was determined. 2 ml of the cell culture were transferred in a tube and centrifuged. Once centrifuged, the liquid was removed with a pipette, and the weight of the remaining wet cell mass was determined.

Figure 3: 2L CONTIBAC® SU that was used in Fig. 9 and Table 2.

Cell Culture	VCD [mio cells/ml]	TCD [mio cells/ml]	Viability [%]	Solid content [g/L]	Crude pH
1	8.56	11.1	77.1	23.5	6.6
2	20.8	22.3	93.2	49.3	6.75
3	27.35	28.19	97.0	58.5	7.15

Table 1: Tested cell culture properties. VCD is the viable cell density, while TCD is the total cell density. The viability is the ratio between the VCD and TCD. VCD = Viable cell density, TCD = Total cell density.

Part of the experiments in the next section were performed at a reduced pH, which was achieved by adding diluted acetic acid. After the pH adjustment (if applicable), purified diatomaceous earth was added to the cell culture. The amount of filter aid is henceforth given as a percentage relative to the wet cell weight (WCW).

Two different types of filter media were used to perform the experiments described in the following section. The first is made of polyethylene having a nominal pore size between 0.8 - 1 µm (henceforth referred to as “fine” filter medium), while the second is made of polypropylene having a nominal pore size of around 6 - 8 µm (henceforth referred to as “coarse” filter medium). Both filter media were manufactured in a GMP compliant environment.

The experiments in the study were performed with two different filtration apparatuses. The results in Figures 5-8 were obtained using a “Nutsche” filter shown in Figure 2. The volume of the Nutsche was 0.2 L, it was operated at a pressure of 1.5 bar, and the filtrate volume was measured in time to determine the filtration rate.

The results in Figure 9 and Table 2 were obtained using a lab-scale 2L CONTIBAC® SU with a filter area of 0.024 m^2 that is shown in Figure 3. The schematic of the test setup is shown in Figure 4. The cell suspension was mixed with filter aid using a FUNDAMIX® model 1 at an amplitude of 1.5 mm and a frequency of 100 Hz. The feed was then pumped in the filter via a Levitronix PuraLev® i100SU pump running at 5’500 rpm. Throughout the filtration, a low pressure of 0.5 bar was used. During the HV filtration, the pressure was increased to 1.1 bar as summarized in Table 2 in the following section. The HV filtration was achieved by pumping compressed air between the filter bag and the filter housing (see Figure 1). After the HV filtration, the cake was washed using a diluted PBS buffer, and WFI was used for back-flush.

To determine the filtrate quality, the turbidity, immunoglobulin

G (IgG), the activity of lactate dehydrogenase (LDH), deoxyribonucleic acid (DNA), and host cell proteins (HCP) were measured. The turbidity is the most classic measurement used to determine the filtrate quality. It is measured by projecting a laser beam onto a 15 ml filtrate sample and determining the amount of scattered light. IgG is a type of antibody and represents the product. The IgG concentration was measured using a Cedex Bio Analyzer from Roche. LDH is an enzyme that is released when cells are damaged. In this study, the activity of LDH was used as an indicator of the cell damage. It was also measured using the Roche Cedex Bio Analyzer. DNA is a molecule containing the genetic information that defines the function of the cell. Similar to the activity of LDH, DNA is released during cell fractures and is considered to be an impurity that is ideally removed during the cell harvest. The DNA concentration was measured using the Invitrogen Quant-iT PicoGreen dsDNA Assay Kit. HCP are impurities that are generated by the cells during the production of biopharmaceuticals and that are ideally already removed during the cell harvest. The HCP measurement was performed using the CHO HCP ELISA Kit 3G F550 from Cygnus Technologies.

Results and Discussion

The first few graphs presented in this study demonstrate the performance of the filtration technology of DrM. Figure 5 shows typical flow rate vs. time curves at a constant pressure. The flow rates, given in terms of liters per square meter per hour (LMH), start very high and drop as the cake resistance increases. Two statements may be made about this result. First, the flow rates are far beyond any regime achieved by conventional SU solid-liquid separation techniques. Second, conventional filters such as depth filters would continue the filtration in Figure 5 until the flow rate drops extremely low, at which point the whole filter is replaced. In the CONTIBAC® SU, on the other hand, the filtration is interrupted once the rate drops significantly and the filter is regenerated. In this manner, a high average flow rate can be maintained throughout the process.

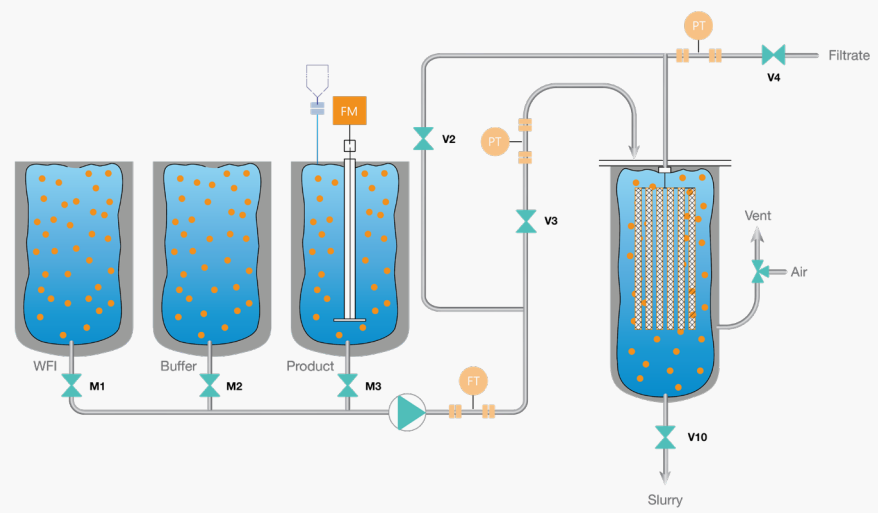

Figure 4: Schematic of the CONTIBAC® SU filtration setup.

From Figure 5 it is also apparent that the pH reduction generally yields higher flow rates, as it agglomerates impurities such as cell debris, DNA, and host cell proteins (HCP) and facilitates

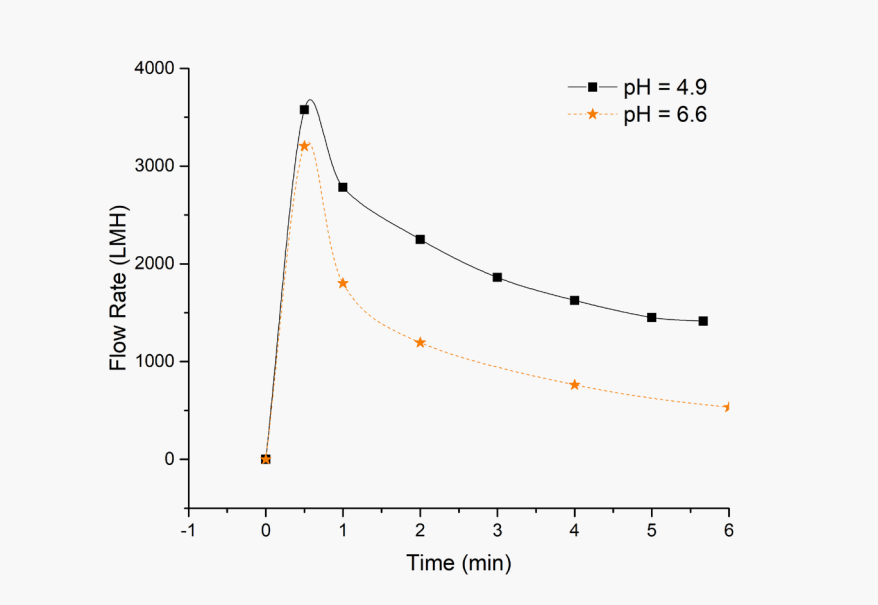

Figure 5: Flow curves at 40% filter aid per WCW. The experiments were performed with cell culture 1 at a pressure of 1.5 bar. The flow rate is given in terms of liters per square meters per hour (LMH).

their separation from the liquid [4].

Besides depending on the cell density, the quality of the crude harvest (viability, LDH), and the pH, the filtration rate also strongly depends on the amount of filter aid. As shown in Figure 6a, the minimum required amount of filter aid is around 20% per WCW. As more filter aid is added, the cake becomes more permeable and the average flow rate increases significantly. Moreover, with an increasing amount of filter aid the benefit of a pH reduction becomes more apparent. At 30 – 40% filter aid per WCW, the flow rates at the reduced pH surpass the flow rates at the crude pH by nearly a factor of five. The performance of the filtration at the crude pH can be increased by switching to a more permeable filter medium having a nominal pore size of 6-8 µm (Figure 6b). Due to the higher porosity, cell debris and impurities do not get trapped in the filter medium. Hence, the flow rates are not limited by the resistance of the filter medium but can be increased by adding more filter aid.

Reducing the pH also allows for maintaining the same average flow rate from one cycle to the next, as shown in Figure 7a. Due to the agglomeration of impurities, hardly any clogging occurs, and the filter medium can be fully regenerated after every cycle. In some situations, however, a pH adjustment is not possible or causes unwanted side effects. For instance, some monoclonal antibodies are susceptible to instabilities and are thus sensitive to changes in the pH [7,8]. Extended exposure of mammalian cells to a low

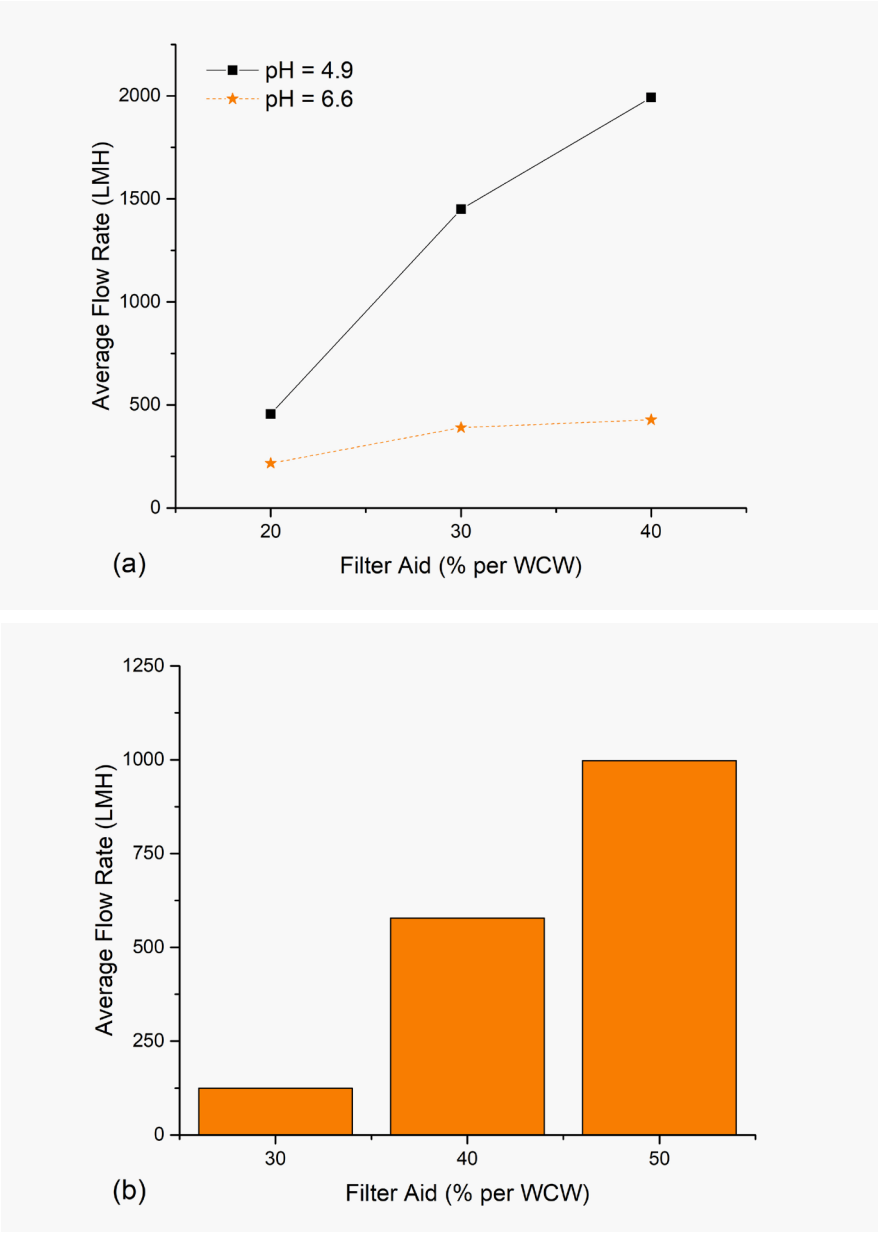

Figure 6: Average flow rates over (a) 200 ml and (b) 100 ml suspension volume, which yielded a cake thickness near 10 mm in both cases. The experiments in (a) were performed with cell culture 1 and the fine filter medium. The experiments in (b) were performed with cell culture 3 and the coarse medium. In both experiments the pressure was set to 1.5 bar.

pH environment can also have adverse effects or even cause cell rupture [9]. In those situations, fouling can still be avoided entirely by choosing a more permeable filter medium with a nominal pore size around 6-8 µm, as is demonstrated in Figure 7b over ten cycles. The results suggest that up to 50 or even 100 cycles can be performed without concern.

The impact of the filter medium permeability on the cyclic behavior at the crude pH is emphasized in Figure 7c. If the fine filter medium is used, the flow rate drops by more than 50% from the first to the subsequent cycle. When using the coarse filter medium, on the

other hand, the flow rate remains unchanged from one cycle to the next. Hence, with the proper choice of filter medium high average flow rates can be maintained with the CONTIBAC® SU that are unrivaled by competing technologies.

The filtration technology of DrM not only demonstrates superior performance, but also produces high filtrate quality. Figure 8a shows that regardless of the pH and the filter aid percentage (within 20-40% filter aid per WCW), turbidities below 15 FNU are achieved. The turbidities achieved are nearly an order of magnitude lower than those achieved in SU centrifugation [10], alleviating the amount of work that has to be performed by subsequent depth or microfilters. With the coarse filter medium, the cake formation takes slightly longer due to the increased pore size, causing a slightly higher initial turbidity. However, the overall turbidities still remain below 20 FNU and can be reduced to 5-10 FNU if the initial few percent of filtrate are either prefiltered, discarded, or if a filter aid pre-coat is used. The activity of LDH does increase as a consequence of the filtration as shown in Figure 8b, however, the increase is much milder than in cross-flow filters [4]. Some of the increase in the activity of LDH can also be attributed to the pH regulation and not the filtration itself, since the pH regulated tests consistently yield higher activity of LDH than tests performed at the crude pH. On the other hand, the pH reduction has the benefit of filtering out nearly all DNA as can be seen from Figure 8c. At both pH values some HCP is filtered out,

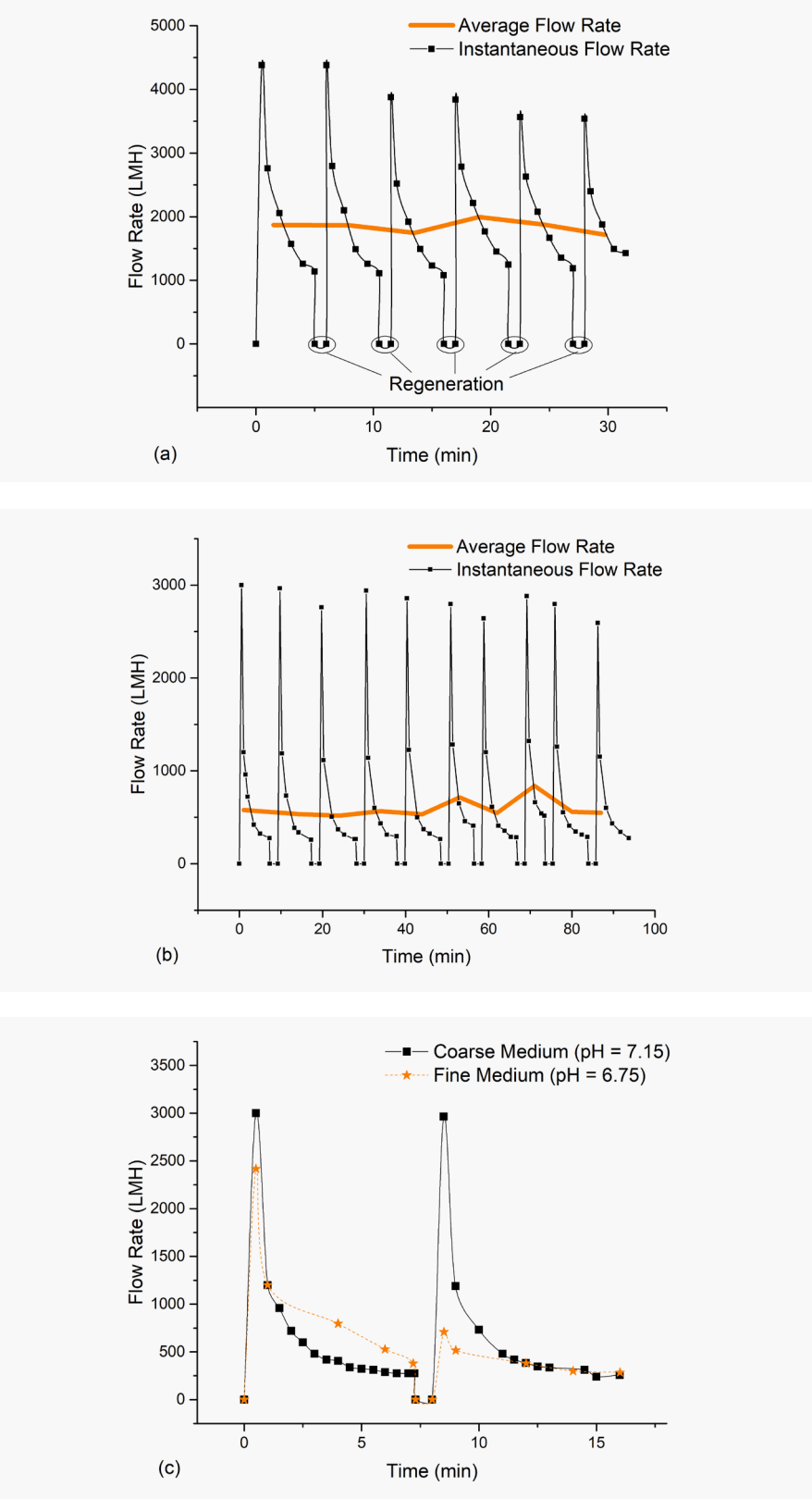

Figure 7: Instantaneous and average flow rates (a) at a reduced pH, performed with the fine filter medium, and (b) at the crude pH, performed with the coarse filter medium. A comparison between the cyclic behavior of the fine and coarse filter media at the crude pH is shown in (c). A pressure of 1.5 bar and 40% filter aid per WCW were used throughout. Experiments with the fine filter medium were performed with cell culture 2, while experiments with the coarse filter medium were performed with cell culture 3.

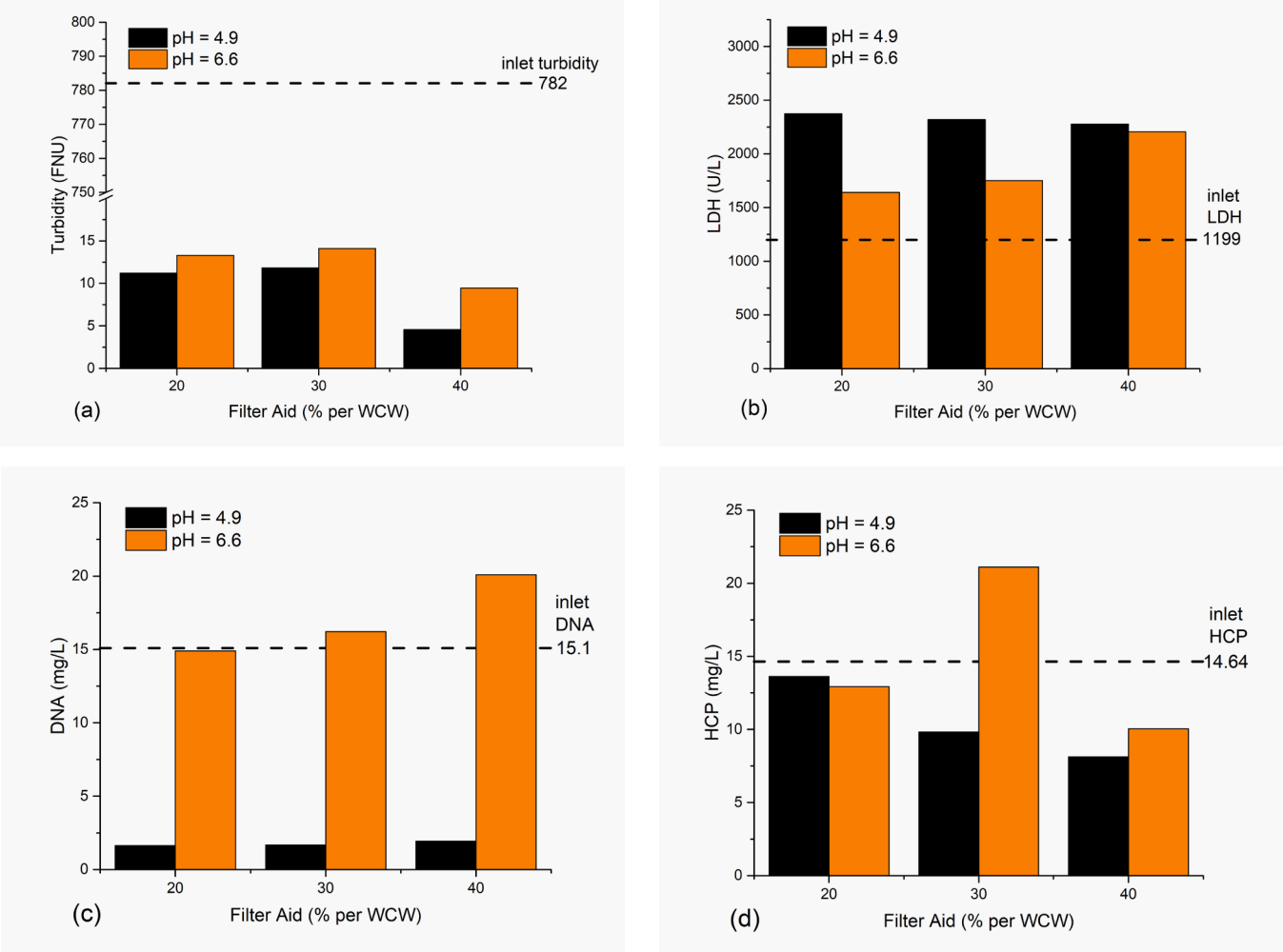

Figure 8: (a) Turbidity, (b) activity of LDH, (c) DNA, and (d) HCP measurements at the crude pH and a reduced pH for 20 – 40% filter aid per WCW. The experiments were performed with cell culture 1 and the fine filter medium. The dashed line shows the corresponding inlet values.

provided the filter aid percentage is beyond 30% per WCW, as shown in Figure 8d.

It can further be shown that the impact of the CONTIBAC® SU filtration system on the product quality is minimal, as is demonstrated in Figure 9. The activity of LDH does not increase much after mixing the cell suspension with the FUNDAMIX® for 30 minutes, pumping it into the filter tank, and running it through the filter medium. It can be concluded that the DrM mixing and filtration technologies induce low shear forces. From Figure 9 it can also be seen that hardly any product (IgG) is lost

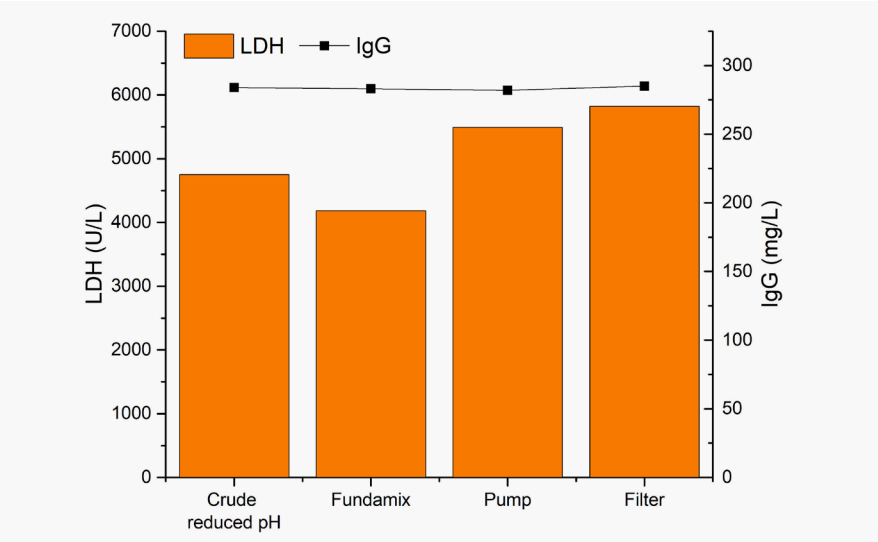

Figure 9: Amount of cell debris (LDH) and product (IgG) measured at different steps of the filtration setup. The tests were performed with cell culture 2, 40% filter aid per WCW, the fine filter medium, and a pH of 4.9. The activity of LDH value after the FUNDAMIX® is lower than the activity of LDH value of the crude harvest due to measurement uncertainties.

	Turbidity [FNU]	Pressure [bar]	Average flow rate [LMH]
Filtration	6.51	0.5	1015
HV Filtration	3.43	1.1	750
PBS wash	12.5	1.1	750

Table 2: Turbidities and average flow rates obtained during the filtration, HV filtration, and the PBS wash, obtained with the fine filter medium.

throughout the various steps of the mixing and filtration process if the quality of the crude harvest is high.

A high filtrate quality can also be maintained throughout the different steps of the CONTIBAC® SU filtration process, as shown in Table 2. The turbidity stays low not only during the filtration, but also during the HV filtration and the PBS buffer wash. Table 2 also shows that even with moderate pressures of 0.5 bar and 1.1 bar, high average flow rates can be maintained during the filtration and HV filtration/PBS wash, respectively.

Conclusions

This study demonstrated that the filtration technology of DrM is not only innovative, but also exhibits exceptional performance while producing high filtrate quality. The high performance of the CONTIBAC® SU filtration technology can be attributed to two revolutionary concepts in the field, namely cake filtration and cyclic operation.

Owing to the cake filtration technique, high average flow rates up to 2'000 LMH can be achieved when reducing the pH and taking advantage of the resulting agglomeration of cell debris and impurities. High flow rates can be maintained over many cycles, even if a rather fine filter medium is used. If a pH reduction is undesirable, high flow rates can be maintained without the occurrence of fouling by using a more permeable filter medium.

The high flow rates of the CONTIBAC® SU do not come at the cost of filtrate quality. It was demonstrated that the turbidity is reduced by up to 98-99%, while only causing a mild increase in the activity of LDH. Moreover, some HCP are removed, and DNA is filtered out almost entirely if a pH reduction is performed.

Due to the cyclic operation of the CONTIBAC® SU, a smaller filter can be used to perform the cell harvest. As a result, the contact area is reduced, along with the amount of leachables and extractables, the footprint, as well as the investment and operating cost. Furthermore, the filtration system excels where conventional filtration technologies crumble, which is at high cell concentrations and large batch volumes.

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