# AXIAL CENTRIFUGAL FILTRATION – A NOVEL APPROACH FOR RAPID BACTERIAL CONCENTRATION FROM A LARGE VOLUME

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## ABSTRACT

A novel approach for filtration on a centrifugal microfluidic platform is presented for the first time. This approach is intended to concentrate bacteria from a large volume. In axial centrifugal filtration the filter is oriented perpendicular to the axis of rotation. This feature allows for integration of dead-end filtration while the filter cake is continuously removed from the filter by centrifugation. This prevents clogging of the filter. Furthermore, a continuous sample feed enables processing of large samples on one disk. Especially for analyzing drinking water large volumes have to be processed and solutions for rapid bacterial concentration are highly appreciated.

## KEYWORDS

Centrifugal microfluidic platform, axial centrifugal filtration, concentration, bacterial enrichment, analysis of drinking water.

## INTRODUCTION

Usually, for bacterial enrichment either centrifugation or filtration is applied. Plain centrifugation requires high centrifugal accelerations larger than 5000 g for concentration of bacteria and in plain filtration the bacteria will be distributed over large areas across the filter. Furthermore, such filters are prone to clogging.

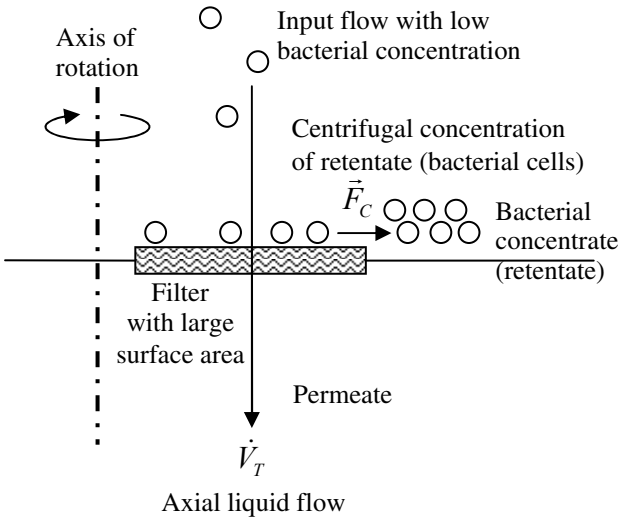


*Figure 1: Schematic of the axial centrifugal filtration approach. An input flow with low bacterial concentration is filtered axially. The retained bacterial cells are centrifuged radially outward to further concentrate the bacteria and to prevent filter clogging.*

Combination of these two approaches, however, enables the application of filters with large cross-sectional areas while the retained bacteria will be centrifuged outward and concentrated further by the centrifugal force (Figure 1). With this combination bacteria can be rapidly concentrated at rotational frequencies at 1000 g even from large starting volumes while preventing clogging of the filter.

## MATHEMATICAL APPROXIMATION

For a first simple mathematical approximation two assumptions are made: the fluid is at rest in the radial direction and the particles to be filtered are spherical in shape. With these two assumptions the radial velocity $v_{rp}$ of the particles can be calculated using the Stokes drag $F_{st}$

$$F_{st} = 6\pi\eta r_p v_{rp} \quad (1)$$

with $\eta$ being the dynamic viscosity of the fluid and $r_p$ the radius of the particle. Furthermore, the centrifugal force $F_c$ on the particles in the fluid is

$$F_c = \tfrac{4}{3}\pi r_p^3 \, \Delta\rho \cdot \omega^2 R \quad (2)$$

with $\Delta\rho = \rho_p - \rho_f$ being the difference in the densities of the particles $\rho_p$ and the surrounding fluid $\rho_f$, $\omega$ the rotational frequency and $R$ the radial distance from the axis of rotation. Of course, the radial velocity of the particle $v_{rp}$ is the ratio of the displacement $dR$ and the time $dt$

$$v_{rp} = \frac{dR}{dt} \quad (3)$$

Combining (1), (2) and (3) and rearranging for $dt$ and integrating from the inner radius $R_i$ to the outer radius $R_o$ yields

$$t = \frac{9\eta}{2r_p^2 \cdot \Delta\rho \cdot \omega^2} ln\frac{R_o}{R_i} \quad (4)$$

This short and simple approximation only allows for a rough estimation of the time required to centrifuge particles off the filter. However, it shows some basic correlations: processing time increases with increasing viscosity of the fluid and/or with increasing desired displacement of the particles. On the other hand, processing time is reduced with larger particles, larger difference in densities of the particles and the surrounding fluid, increased rotational frequency and/or increased radial distance from the axis of rotation.

## MICROFLUIDIC LAYOUT

The novel unit operation for axial centrifugal filtration was realized by integration of a filter into a microfluidic structure which has been outlined on both sides of a LabDisk [1] (Figure 2). A radially inward positioned sample inlet chamber on the upper side of the disk is connected to a filter chamber harboring an integrated filter. Here, the sample has to pass through the filter and a through-hole in the disk to the lower side of the disk. A permeate chamber on the lower side then collects the permeate.

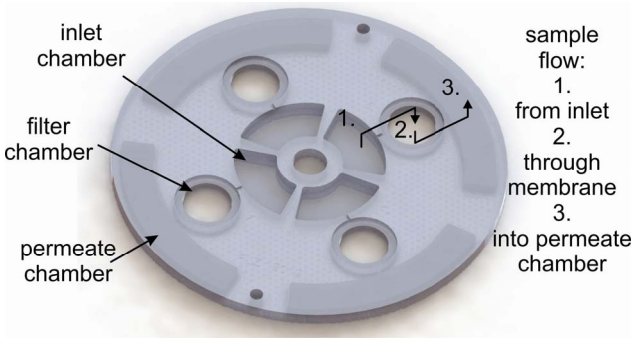


*Figure 2: Microfluidic structure for on-disk enrichment of bacteria. The novel unit operation consists of three chambers: an inlet chamber at the upper side of the disk into which the sample is loaded, a filter chamber connecting the upper side of the disk to the lower side and a permeate chamber on the lower side collecting the permeate of the filtering process. Four enrichment structures fit on one LabDisk.*

## FABRICATION

The microfluidic structure shown in Figure 2 has been milled by the HSG-IMIT Lab-on-a-Chip Design- and Foundry Service, Freiburg, into a PMMA substrate. After the milling process a polycarbonate track etch filter with pore size of 0.2 µm (Nuclepore 800281, Whatman International Ltd., United Kingdom) was inserted into the filter chamber and bonded to the PMMA substrate at a temperature of 140 °C and at a pressure of 7.6 bar for a process duration of 10 s. Then, both sides have been laminated with adhesive foil (900360-S, HJ Bioanalytik GmbH, Möchengladbach, Germany) to seal the microfluidic structures.

## EXPERIMENTAL EVALUATION

In initial tests, the leak-tightness of the filters has been demonstrated. After loading a sample of up to 500 µl per structure, the disk was rotated for 2 min at 80 rotations per second (corresponding to a centrifugal acceleration of 1000 g) to centrifuge the sample through the filter. Particles and bacteria remained on the filter and have been enriched during the process.

The functionality was first tested using magnetic beads (ajInnuscreen, Berlin, Germany) with a diameter of 250 nm as these beads provide a strong contrast and in agglomerates are easily visible to the naked eye. The beads could successfully be retained and enriched using the on-disk filter in 4 out of 4 experiments.


*Figure 3: Photograph of the produced LabDisk carrying the axial filtration structures. Filters are inserted into the filter chambers by thermal bonding. The LabDisk is inserted into a receptacle which can be used to collect clean and filtered permeate when processing samples of large volumes. The receptacle can be connected to a larger vessel for storage of the larger fluid volumes.*

In a second test, bacterial suspension was filtered and enriched on the disk. A dilution series of *E. coli* culture [2] containing $10^8$, $10^7$, $10^6$ and $10^5$ cfu · $ml^{-1}$ was loaded onto the LabDisk (Figure 3) and centrifuged through the filter. The liquid contained in the permeate chambers was plated on plate count agar plates (VWR, Bruchsal, Germany) and incubated overnight at 37 °C. None of the plates showed any bacterial growth in 12 out of 12 runs, i.e. bacterial reduction by filtration was at least $10^8$. Bacteria collected from the filter cake however could successfully be cultured indicating that the on-disk enrichment sustains viability of the bacteria (Figure 4).

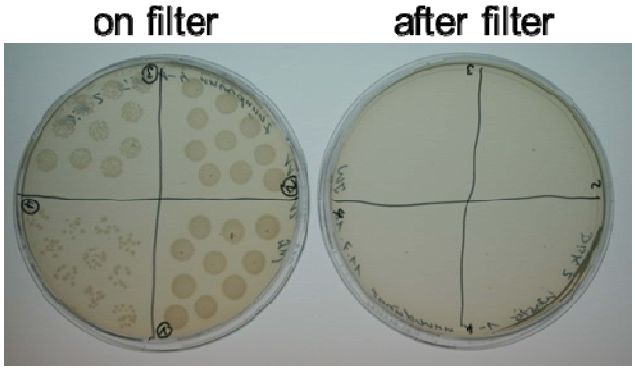


*Figure 4: After on-disk bacterial filtration, the retentate (on filter) as well as the permeate (after filter) have been plated on PC agar plates and incubated. Bacteria from the retentate could successfully be cultivated whereas the permeate was completely free of bacteria.*

## RE-DESIGN FOR PROCESSING LARGE VOLUMES

To enable processing of larger sample volumes the four inlet chambers were merged to form just one inlet chamber which was arranged on the circumference around the axis of rotation. This design allowed for

continuous [3] non-contact [4] application of sample onto the disk and for processing of much larger sample volumes than the volume of the inlet chamber. A square form of the inlet chamber with the corners pointing towards the four filter chambers automatically divided the sample into four aliquots to be processed on the four filters respectively. To remove the processed fluids from the disk (also large volumes), a channel directed radially outward with an open end at the rim of the disk was included on the lower side of the disk. Thus, the processed fluid was centrifuged off the disk. Permeate chambers have been omitted (Figure 5) in this design as clean permeate has been collected off-disk with a receptacle (Figure 3). Furthermore, additional collection chambers positioned radially outward from the filters have been added into which the particles and bacteria retained by the filter were centrifuged.

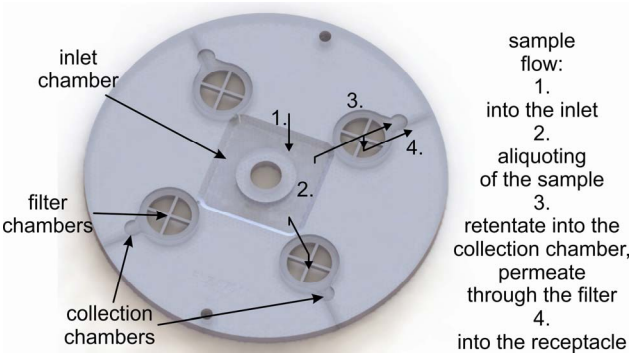


*Figure 5: Microfluidic structure for continuous on-disk axial filtration of bacteria featuring one inlet chamber arranged circularly around the axis of rotation. A filter support structure is implemented inside the through holes of the filter chambers. Due to the centrifugal force, bacteria are transported radially outwards, across the surface of the filter into collection chambers. Permeate is continuously transferred to the receptacle.*

To evaluate processing of large volumes, di-water was continuously applied onto the disk using a peristaltic pump (Ismatec MCP Process IP65, IDEX Health & Science, Wertheim, Germany). A through-put of 5 l in 38 min corresponding to a volume flow rate of 2.2 ml · $s^{-1}$ at a rotational frequency of 80 rotations per second has been demonstrated.

After having processed 5 l of di-water with the disk the leak-tightness was investigated by application of 2 ml of *E. coli* suspension in Liquid Broth at a bacterial count of $1 \cdot 10^8$ cfu · $ml^{-1}$. The *E. coli* suspension was processed at a rotational frequency of 80 rotations per second (i.e. 1000 g) for 2 min. Due to the centrifugal force bacteria were centrifuged radially outward into the collection chambers preventing clogging of the filter. Samples have been taken from the collection chambers and the filters. Furthermore, the permeates have been collected (disk design including permeate chambers, not shown). To determine the bacterial concentration each sample has been plated and incubated overnight at 37 °C.

The filters withstood the harsh conditions of continuous processing at 80 rotations per second for 38 min. However, the filters were not as leak-tight as in the experiments with only 500 µl of *E. coli* suspension. Out of the 8 permeates 5 were completely free of bacteria. The other permeates contained only 10, 30, and 1300 cfu · $ml^{-1}$, respectively. However, considering the starting concentration of $1 \cdot 10^8$ cfu · $ml^{-1}$, leakeage is extremely small since the concentration was reduced by roughly 5 orders of magnitude.

Comparison of the bacterial count of the samples taken from the collection chambers with the samples taken from the filters yields a 24-fold enrichment of *E. coli* in the collection chambers. Thus, bacteria have indeed been centrifuged into the collection chamber.

Using equation 4, the time required for centrifugation can be estimated: With the parameters $\eta = 1$ mPa · s, $r_p = 1$ µm, $r_p = 1.08$ g · $cm^{-3}$ [5], $r_f = 1.0$ g · $cm^{-3}$, $\omega =$ 500 Hz, $R_i = 35$ mm, and $R_o = 48$ mm the equation yields a centrifugation time of $t = 70$ s. Hence, to further increase the enrichment factor at the same rotational frequency the sample should be processed for a longer period of time due to the low volume flow rate.

## CONCLUSION

In conclusion, the novel unit operation for axial centrifugal filtration successfully filters and enriches bacterial suspensions while sustaining the viability of the processed bacteria. The structure features leakage-free filtration at low volumes in the ml range. The centrifugal force continuously removes particles and bacteria form the filter in the radially outward direction, thus avoiding clogging of the filter. In addition, the concentration of the particles and bacteria in the retentate is even increased by this motion. Moreover, this approach allows for continuous sample input for processing large volumes in the liter range or even larger. Potential fields of application include all filtering processes particularly monitoring of water contaminations.

## OUTLOOK

For processing large sample volumes of 5 l and more the filters need to be stabilized to prevent leakage. This can be implemented by optimization of the filter support structures and the bonding process.

Furthermore, with axial centrifugal filtration available, this novel unit operation should be integrated with downstream unit operations for further processing of the retentate. For instance, when filtering water samples bacteria in the retentate could be genotyped via lysis, DNA extraction and qPCR analysis. Such an integrated disk would be highly appreciated as it does not only quantify the bacteria in the sample but also determines the species contained in it.

## ACKNOWLEDGEMENTS

The present study is funded by AiF within the project KomPro (16583 N)

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