Cell debris removal by single-use diatomaceous earth (DE) filtration

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Introduction

Many biotechnological products are obtained by the help of native or recombinant microbial cells in fermentation processes. In order to get intracellular target molecules, like proteins or enzymes, the cells have to be destructed. This is done with cell rupture techniques (e.g. high-pressure homogenization) which generate a suspension containing cell debris and cell contents (proteins, DNA, mRNA, etc.). The first step in the following purification sequence is the removal of the cell debris. One possibility to do this is the usage of depth filters like the FILTRODISC™ BIO SD filter capsules from FILTROX. Often, a removal of cell debris is not possible by sole use of such filter capsules because of filter blocking. Therefore they are usually used for so-called body-feed filtrations, in which a filter aid like diatomaceous earth is mixed with the unfiltrate to improve the filtrate flow by a decreased cake compressibility and an increased cake permeability. To enhance the separation further an additional precoat layer consisting of the filter aid could be applied (see figure 1).

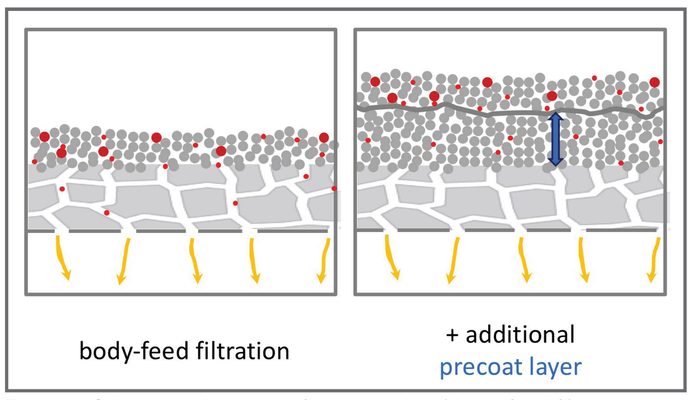

Figure 1: Schematic diagrams of a conventional body-feed filtration and a body-feed filtration with an additional precoat layer

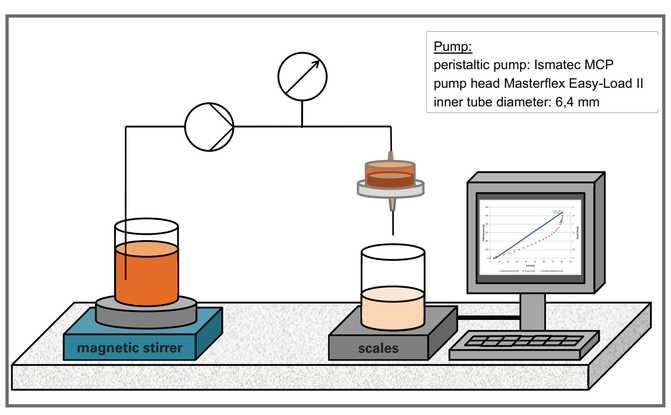

Figure 2: Experimental set-up

Experimental set-up

Filtration experiments were done to remove cell debris of *E.coli* which produces a recombinant enzyme. A phosphate buffer (pH = 7.5) was used to maintain the enzymatic activity. A cyclic process in a high pressure homogenizer ($\Delta p \approx 1000$ bar) ensured that more than 99 % of the cells were destructed. The cell concentration before rupture was 25 g/L (wet biomass). Figure 2 shows the experimental set-up. The unfiltrate containing the filter aid Celpure® C65 was mixed by a magnetic stirrer during the whole filtration primarily to avoid sedimentation. It was pumped into the filter capsule by the help of a peristaltic pump at a set point of 4.43 L/(min·m²). The filtrate mass was measured automatically and time dependent with a scales and a computer. The quality of the obtained filtrates was determined by measurements of the optical density and the protein concentration (Bradford-test). Therefore samples of the filtrate were taken in regular time intervals. The optical density at a wavelength of 600 nm (OD_{600}) is a parameter to determine turbid materials like cells or cell debris.

Comparison body-feed filtration with and without precoat layer

Figure 3 shows the developments of the optical density and the protein concentration of the filtrates during three filtration set-ups. If the same filter capsule (CH 153 P) is used, an additional precoat layer reduces the OD_{600} noticeable in comparison to a conventional body-feed filtration. When the filter capsule CH 145 ZP is applied, it is even possible to keep the OD_{600} below 0.01. At the same time the protein concentration of each filtration rises directly at the beginning and reaches a constant value. Therefore the flux of the enzymes through the filter cake, the precoat layer and the filter media is not hindered in a visible way.

To rate the measured values of the OD_{600}, one should consider that the shoulder of the absorption band of enzymes (maximum at 280 nm) falsify the measurements of the OD_{600}. Consequently, the measured values are not only caused by cell debris but also by the cell contents. This implies that almost all cell debris were removed when an additional precoat layer was applied. The disadvantage of this method is the higher amount of filter aid, which could lead to a higher adsorption of the target molecules. Executed adsorption experiments verify this effect in the regarded case.

Figure 4 compares the developments of the filtrate mass and the pressure of a body-feed filtration and a body-feed filtration with an additional precoat layer by using the same filter capsule (CH 145 ZP). The filtrate mass rises linearly in both cases till the end of the filtration. The effective volume flows are slightly below the set point because of an additional pressure drop caused by the filter cake and the rising blocking of the filter media. The precoat layer reduces this pressure drop by avoiding the blocking of the filter media. So the filtrate flux is here 14 % higher. But the pressure in these experiments rises in a different way. When a precoat layer is applied, the slightly higher increase at the beginning is caused by the precoat layer which was formed before the real filtration starts. But afterwards the pressure rises slowly and linear. In contrast the pressure in the conventional body-feed filtration reaches faster the maximum allowed pressure.

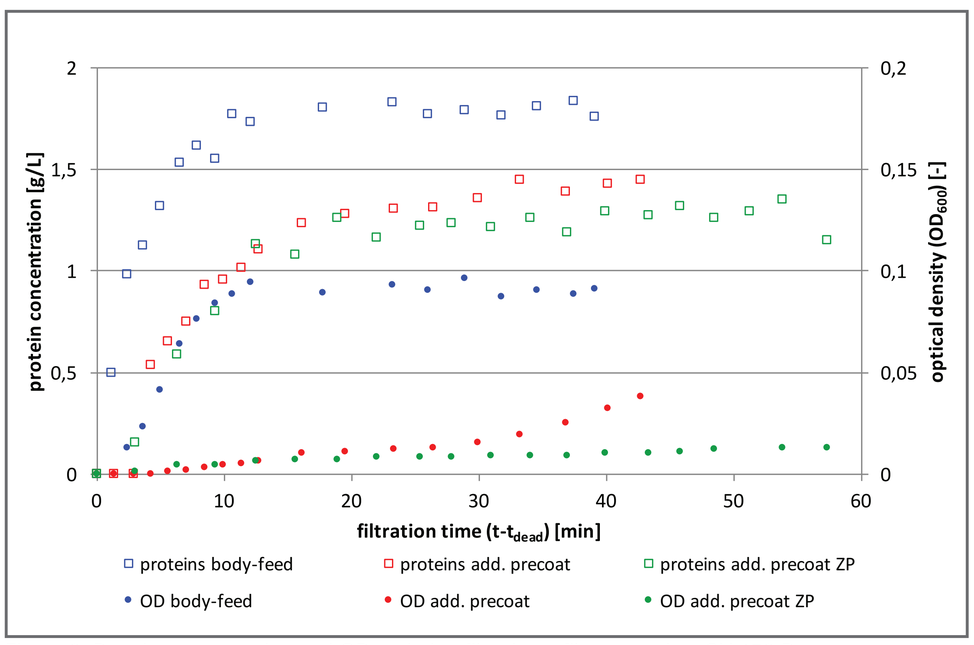

Figure 3: Protein concentration and OD_{600} during filtrations, filter capsules: CH 153 P and CH 145 ZP, concentration of ruptured cells: 25 g/L, filter aid: Celpure® C65, concentration filter aid: 25 g/L, precoat: 5 g

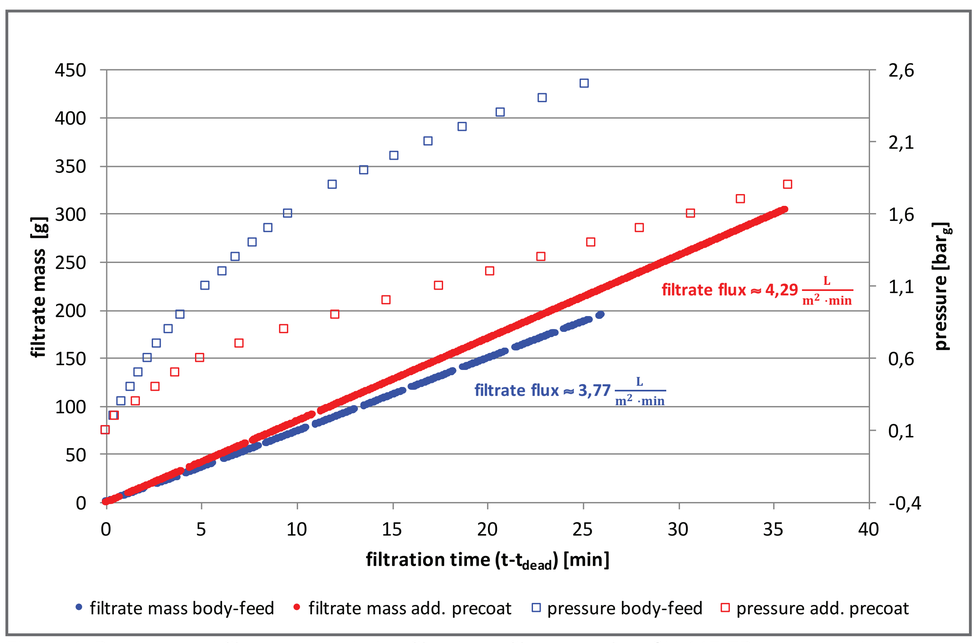

Figure 4: Pressure and filtrate mass during filtrations, filter capsules: CH 145 ZP, concentration of ruptured cells: 25 g/L, filter aid: Celpure® C65, concentration filter aid: 25 g/L, precoat: 5 g

Conclusion and outlook

The advantages of an additional precoat layer in the body-feed filtration are a lower pressure increase in combination with a higher filtrate flux. Furthermore the precoat layer leads to an enhanced removal of cell debris. One disadvantage is surely the loss of target molecules (e.g. proteins) due to adsorption on the filter aid. An opportunity to get more target molecules could be a rinsing process after filtration, which will be tested in further experiments. Nevertheless a body-feed filtration with an additional precoat layer is an excellent method to separate proteins and cell debris. Especially because the removal of cell debris using FILTRODISC™ BIO SD and Celpure® C65 can be realized without further addition of flocculants and without changing the pH value.