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Partner in Research

Application of novel hollow fiber micro-bioreactor to integrated downstream processes

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Introduction

Hollow fiber micro-bioreactors are used in different fields of application in biotechnological processes, e.g. classical filtration processes, cultivation of different test organisms, immobilization of enzymes as biocatalysts (enzyme micro-bioreactor) and product purification. The purification of target molecules (metabolites, proteins) can be achieved by using convenient modified hollow fiber membranes. For high-throughput experiments an arrangement of several micro-bioreactors in parallel can be realized. Furthermore, a combination of cultivation technology and following downstream processes (integrated downstreaming) offers a great potential in microbiotechnology. The miniaturization of the reactor system as well as the integration of operation steps positively influence the time- and cost-intensive downstream process.

The broad application of hollow fiber micro-bioreactors in various areas of biotechnology is still restricted due to a limited functionality of the membranes. With this work a straight forward procedure for membrane functionalization followed by their utilization in downstream processes is presented.

Modification strategy

First of all, PES/PS hollow fibers were modified by reacting terminal hydroxy groups with diepoxide ethylene glycol diglycidyl ether (EGDGE). For increasing loading capacity hydroxyethyl cellulose (HEC) polymers were bound to the epoxy groups (step 2). Second epoxidation produced final polymers containing reactive epoxy groups on the hollow fiber surface (step 3, basic activation). Reaction step 4 is referred to the immobilization of IDA for producing hollow fibers with chelating properties. Finally, divalent metal ions (Cu^{2+}, Ni^{2+}, Co^{2+}, Zn^{2+}) were coordinated to the bound chelator.

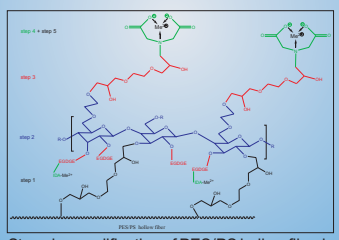

Fig. 1: Stepwise modification of PES/PS hollow fiber hydroxy end groups to produce IMAC affinity membranes.

Loading capacities

Table 1 shows a binding capacity between 0.33 and 0.56 µmol of EGDGE / g hollow fiber in relation to used fiber material in the first reaction step. After linking HEC (step 2) to hollow fiber surface and second epoxidation (step 3) the concentration of bound epoxy end groups significantly increases. Loading capacities between 7.03 and 9.88 µmol epoxide / g fiber were achieved. The reaction sequence illustrates how functionality of these hydrophobic fibers can be amplified. In comparison to first epoxidation the epoxide concentrations after amplification increase by 13-22 fold. Amplification of reactive hydroxy end groups is based on covalently linked HEC.

Table 1: Loading capacities of bound epoxide groups and HEC polymers (basic activation, steps 1-3).

Hollow fiber material (pore size)	Loading capacity [µmol Epoxide or mg HEC / g hollow fiber]			
	Step 1	Step 2		Step 3
	µmol Epoxide	µmol Epoxide	mg HEC	µmol Epoxide
MicroPES (0.2 µm)	0.46 ± 0.09	0.09 ± 0.13	11.8 ± 2.3	8.59 ± 1.15
UltraPES (70 kDa)	0.33 ± 0.12	0.12 ± 0.04	9.78 ± 0.35	7.35 ± 0.55
High Flux PS (30 kDa)	0.56 ± 0.07	0.23 ± 0.19	9.9 ± 2.3	7.03 ± 0.92
Low Flux PS (5 kDa)	0.44 ± 0.07	0.19 ± 0.19	11.2 ± 2.3	9.88 ± 0.93

Purification of GFP-His_6 from *E. coli* with IMAC membranes

The effect of different chelated metal ions (Cu^{2+}, Ni^{2+}, Co^{2+} and Zn^{2+}) on adsorption capacity of GFP-His_6 was investigated with MicroPES and UltraPES IMAC hollow fiber membranes. The Me^{2+}-IDA-membranes presented a metal saturation capacity in the lower µmol range per g dry hollow fiber. In order to estimate the best metal ion, preliminary adsorption experiments were carried out using imidazole as a competitive agent (single step elution). Protein and GFP-His_6 binding efficiency were determined by two different ways (differentiation and elution values). In relation to the elution values the strenght of adsorption of his-tagged GFP decreases in the following order: Cu^{2+}/Ni^{2+} > Zn^{2+} > Co^{2+} (shown in Table 2).

Furthermore, it is also important to analyze the selectivity of the adsorption process. Fig. 2 shows SDS-PAGE analyses of each purification step of the downstream process of GFP-His_6 with UltraPES IMAC hollow fiber membranes. The order for the selectivity of adsorption of GFP-His_6 onto immobilized metals is contrary in relation to the binding efficiency: Co^{2+} > Zn^{2+} > Ni^{2+} > Cu^{2+}.

Table 2: Metal-bonding capacities of IMAC hollow fiber membranes and their sorption capability for GFP-His_6.

IMAC hollow fiber membrane	Loading capacity				
	Metal concentration [µmol Me^{2+} / g hollow fiber]	Protein concentration [a] [mg protein / g hollow fiber]		GFP-His_6 concentration [b] [mg GFP-His_6 / g hollow fiber]	
		Differentiation values [c]	Elution values	Differentiation values [c]	Elution values
MicroPES-Cu^{2+}	4.04 ± 0.22	43.57	5.81	5.45	3.01
MicroPES-Ni^{2+}	18.39 ± 2.92	18.13	3.03	5.58	1.96
MicroPES-Co^{2+}	14.12 ± 1.31	12.87	0.21	4.87	0.04
MicroPES-Zn^{2+}	-----	26.90	0.31	4.83	0.04
UltraPES-Cu^{2+}	5.82 ± 0.22	36.55	1.66	5.05	1.14
UltraPES-Ni^{2+}	14.01 ± 1.46	19.88	2.82	5.36	1.84
UltraPES-Co^{2+}	11.50 ± 1.31	35.67	0.32	4.65	0.22
UltraPES-Zn^{2+}	-----	32.16	1.95	4.55	0.89

a Protein concentrations were determined by Bradford assay.
b GFP-His_6 concentration was directly determined by fluorescence memasurement (main peak, excitation at 395 nm and emission at 510 nm).
c Differentiation between protein (GFP-His_6) concentrations of loading solution and supernatant.

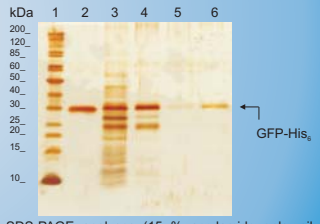

Fig. 2: SDS-PAGE analyses (15 % acrylamide gels, silver stained). Lane 1: Marker, Lane 2: rec. GFP (positive control), Lane 3-6: 250 mM imidazole elution fractions (3 = Cu^{2+}, 4 = Ni^{2+}, 5 = Co^{2+}, 6 = Zn^{2+}).

Hollow fiber micro-bioreactor

Fig. 3 shows different hollow fiber micro-bioreactors. The module casing consists of a transparent plastic housing. Utilization of hollow fibers effects a separation of the reactor system into 2 reaction spaces, named extracapillary and intracapillary space. Due to reactor type the volume ranges from 30-40 mL.

An improvement of the reactor system for cultivation processes was achieved via the integration of a stirrer and an additional layer of fibers for oxygen supply (PVDF). Media supply is realized by a layer of fibers made from UltraPES (Fig. 3a). Fig. 3b shows an affinity reactor module which consists of one layer of Co^{2+}-functionalized UltraPES hollow fiber membranes.

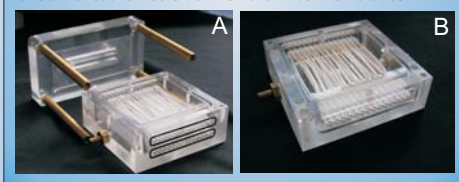

Fig. 3: Hollow fiber micro-bioreactor for cultivation processes (A) and affinity micro-bioreactor system (B).

Integrated downstream processes

For realizing integrated downstreaming cultivation of *E. coli* (GFP-His_6, intracellular product release) was performed in a hollow fiber micro-bioreactor (30 °C, 300 rpm, 4 mL/min). Fig. 4 shows the growth curve with lag phase, exponential phase and the change over to a stationary phase. In addition, GFP fluorescence in the cells during cultivation was determined.

After cultivation process culture broth was removed and cell lysis was achieved by repeated freeze-thaw cycles. For purification step affinity micro-bioreactor modules were loaded with unclarified samples. To assure high selectivity during purification process of GFP-His_6 Co^{2+}-functionalized modules were used. Elution steps (1-3) were carried out using only the intracapillary space because of taking advantage of the separation of reaction spaces as well as the utilization of small elution volumes (5 mL). The results of integrated downstream processes are shown in Table 3.

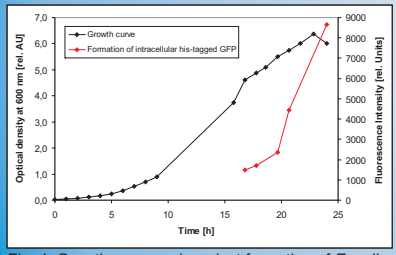

Fig. 4: Growth curve and product formation of *E. coli* (GFP-His_6).

Table 3: Sorption capability for GFP-His_6 of different affinity micro-bioreactor modules used in integrated downstream processes.

Affinity micro-bioreactor module (number of hollow fibers)	Loading capacity [mg GFP-His_6 / g hollow fiber]				
	Differentiation values [a]	Elution 1	Elution 2	Elution 3	Σ Elution [b]
MicroPES-Co^{2+} (150)	1.12	0.14	0.05	0.02	0.21
UltraPES-Co^{2+} (43)	1.24	0.09	0.06	0.05	0.20
High Flux PS-Co^{2+} (300)	1.19	0.04	0.03	0.02	0.09
Low Flux PS-Co^{2+} (200)	3.03	0.17	0.07	0.07	0.31

a Differentiation between GFP-His_6 concentrations of loading solution and supernatant.
b Sum of elution values 1-3 (intracapillary elution process).

Summary & Outlook

This work shows the possibility to functionalize commercially available PES and PS hollow fiber membranes with different reactive ligands. Starting from a limited number of hydroxy groups on hollow fiber surface it was possible to convert these hydrophobic membranes into hydrophilic ones. On the basis of epoxidized fibers it is possible to couple IDA followed by coordination of divalent metal ions to generate IMAC membranes.

Here, we have successfully demonstrated that IMAC hollow fiber membranes made of PES/PS are a potential alternative for the rapid purification of his-tagged GFP. In relation to purification strategies of GFP-His_6 Co^{2+} is used to achieve high selectivity while the application of Cu^{2+} and Ni^{2+} provides predominantly high binding capacities. Furthermore, utilization of novel hollow fiber micro-bioreactors for cultivation as well as for purification processes was achieved.

From this and other work in progress we want to optimize final coupling reaction steps. In addition, next targets are the application of micro-bioreactor system for purification of extracellular his-tagged proteins produced by *Bacillus megaterium* (Levansucrase-His).

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