A NEW FILTER APPARATUS FOR SELECTIVE BIO SEPARATION

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ABSTRACT

One of the emerging fields in downstream processing of the modern biotechnology lies in the use of particulate systems with functionalized surfaces to separate a target bio product like protein, enzyme, DNA etc. from bio broth by selective adsorption. Especially here the application of magnetic separation methods by using functionalized magnetite seeded polymer particles has emerged strongly for analytical purposes. To apply the same process concept to industrial bio production processes new technologies have to be provided which allow effective, reliable and economical service.

The combination of two classical separation mechanisms, cake filtration and magnetic separation, described in this paper, results in positive synergetic effects and an extension of the field of application of the cake filtration process into selective bio separation.

KEYWORDS

Bio separation, Cake filtration, Magnetic separation, Magnetic filtration, Selective Adsorption

1. Introduction

As the global market for so called nanomagnetics is supposed to rise from 4.3 billion$ nowadays to approximately 12 billion$ by the year 2009 [BC Comp. Inc.] the attractiveness of process extension especially by application of magnetic fields raises equally. Nanomagnetics have a wide range of use in life sciences and in electronics industry for the production of storage media. Especially in life sciences the application of surface functionalized magnetically seeded polymer particles is establishing in diagnostics, cancer treatment and bio separation. Diagnostical usage of surface functionalization has already had its break through in DNA, cell separation, etc.. Most applications usually need little amounts of functionalized particles, for which one reason is the comparatively high production costs and the lack of process machinery capable for the use within magnetic fields and with the appropriate capacity. Recent research is therefore especially aiming at the development of technologies that allow the processing of these particle systems at high throughputs for a specific target product separation.

The basic principle of modern biotechnological processes is the use of natural metabolism of an organism to produce complex molecules during fermentation. This enables the development of new products such as pharmaceuticals as well as the cost-effective production of known products. However beside the desired product a fermentation broth usually contains also several side products. These have to be removed in time and money consuming purification steps, the so-called downstream

processing. It consists of different unit operations such as chromatography, ultra-centrifugation, filtration, and many more. Up to 80% of the investment and operating costs originate from this downstream processing. If one considers that in the year 2005 more than 50% of newly licensed medicaments originate from a biotech facility and the predictions assume that in 2018 more than 50% of all offered medicaments are produced in a bio process, the usage of specific separation processes gains high importance to overcome product loss as well as bottlenecks in actual downstream processes. By using highly functionalized magnetic substrate particles, which adsorb the desired product like protein, enzyme, DNA etc. selectively, the product can be separated out of a mixture of non magnetic components, e.g. bio broth. A wide range of these surface ligands for different target products are already in application, e.g. in diagnostics or in adsorption chromatography.

2. Magnetic separation in the downstream processing

The separation process consists of the following phases: fermentation, mixing, specific adsorption, magnetic separation, washing and elution (Fig.1).

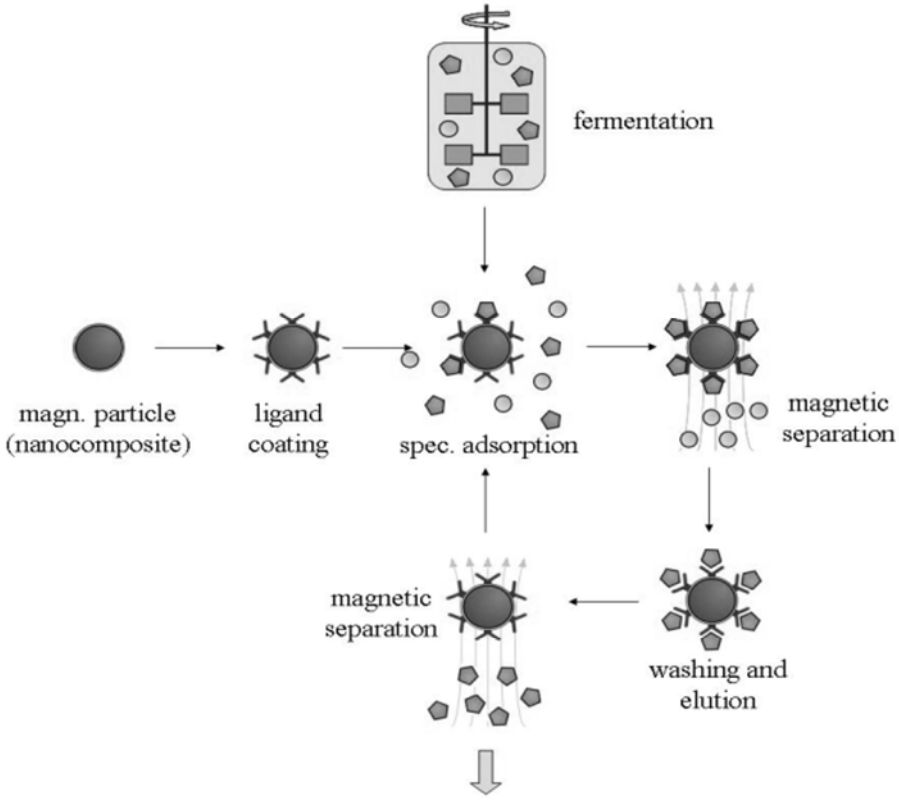

Fig.1: Process schema of the magnetic selective bio separation

First the magnetic carrier particles, reusable in several separation cycles, have to be produced and functionalized in a pre process. Then the product-specific functionalized particles are mixed with the fermentation broth. Depending on the explicit fermentation environment a precipitation or similar pretreatment may be necessary. The mixing provides intense contact of the particles with the bio broth so that mass transfer limitations do not have to be considered. The separation of the carrier particles with the attached product now depends on the magnetic properties of the carrier particle rather than on the actual bio product properties. After the first magnetic separation the particles are washed several times to reach the desired

purity. Normally during washing the particles are redispersed into washing liquor. To remove the contaminated washing liquor, other magnetic separation steps are performed. To retrieve a pure solution of the final product, it is detached from the particle surface in an elution step. This elution is realized by a change of pH, ionic strength, temperature or similar, depending on product and binding properties. The carrier particles can be used in the next separation cycle. The advantage of magnetic separation over classical chromatographic technologies is the higher capacity of the particles due to their smaller sizes and better product contact, maximal product recovery, minimal separation time, and the reduction of unit operations at the same time.

The magnetic separation step can be realized in different ways. Investigations mainly focus on the High Gradient Magnetic Separation (HGMS) which uses a magnetic matrix, comparable with a very loose deep bed filter, to achieve high field gradients and thus high magnetic forces. Compared with HGMS the magnetic field enhanced cake filtration features a higher cleanability of the nutsche filter which is of importance regarding hygienic design aspects and suspensions with higher solid concentrations can be handled. Advantageously is especially the merging of a whole process, as depicted in Fig.1, in only one apparatus. Also the production of the magnetic particles can take place in the apparatus.

3. Methods and Materials

Fig.2 shows the scheme and a photo of the experimental apparatus. A non magnetic nutsche filter (Bokela GmbH, Germany) with a filtration area of 50cm^3 is immerged in the bore of a solenoid. The filter media (Filtryl Z-1500-K015 (ZBF, Germany)) has an average pore size of 15µm. In the experiments a filtration pressure of 0.8bar is applied. The electro magnet (Steinert Elektromagnetbau GmbH, Germany) has maximal field strength of 0.4T, an iron frame bundles the magnetic field inside the bore. The filter cell is positioned a way that the highest magnetic forces occur in the region of the filter media. In this position magnetic and pressure forces are acting in opposite directions which enables the prevention of a filter cake built-up for high field strengths. Due to a radially acting magnetic field gradient the particles are not only directed counter wise to the filtration direction but also to the side of the filter cell. Either way the filter media is kept free of particles. Two minutes prior to each separation step the magnetic field is applied to give the possibility for the particles to move away form the filter media. A stirrer is integrated to provide redispersion of the deposited magnetic particles. This is supported by a wash nozzle which allows the removing of the particles of the inner wall of the nutsche.

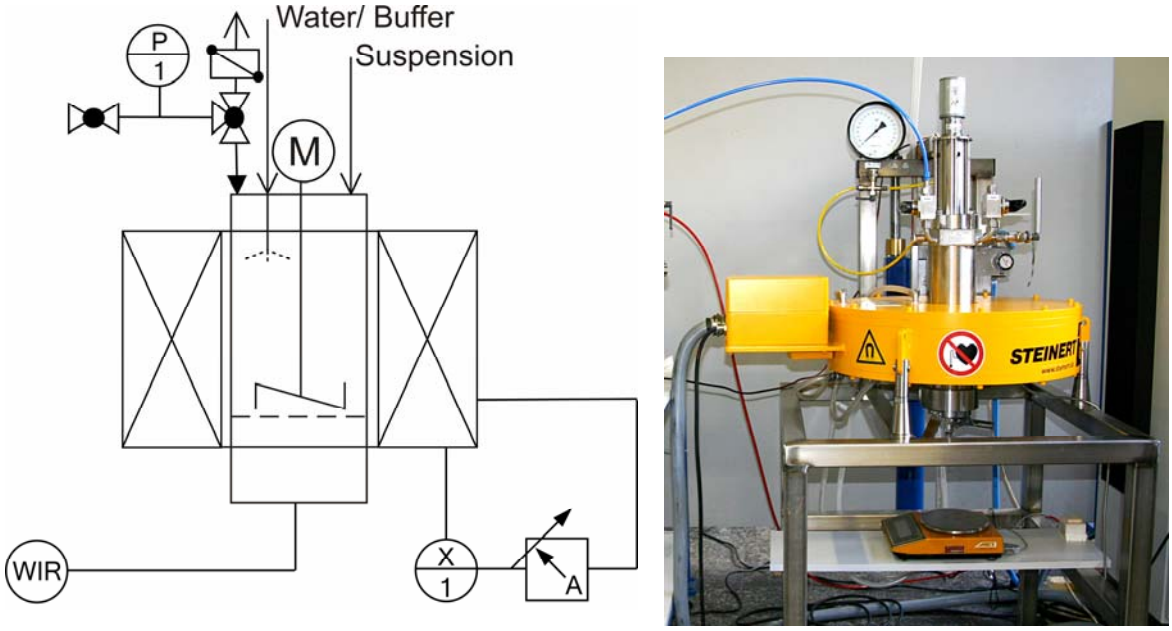

Fig. 2: Scheme and photo of the experimental setup

For the experiments magnetite seeded polyvinyl acetate particles (PVAc) are used with a cationic ion exchanger surface functionalization. The beads do not have a remanent magnetization and do not agglomerate after turning off the magnetic field. This has advantage in adsorption and elution steps as well as in the reusability of the particles in further cycles. As a model target product the enzyme lysozyme is used without further side components. The exact properties of the magnetic beads and the lysozyme are listed in Tab. 1.

Tab.1: Product properties

			Magnetic Beads	***Lysozyme***
Particle Size	d_{50}	[µm]	4.49	0.01
Density	ρ	[g/cm³]	1.47	
Mol weight		[g/mol]		14388
Magnetization	M_s	[Am²/kg]	22.56	-
	M_r	[Am²/kg]	0	-
Potential (pH=7)	ξ(slurry)	[mV]	-36	-10
Feed concentration		[g/l]	10	2

For the different adsorption and elution steps the pH-value is adjusted exactly. The used buffer solutions are listed in Tab.2.

Tab.2: Buffer Solutions

	pH	**buffer**	**addition**	**time to equilibrium**
Adsorption	8	0.20 mmol phosphate buffer (NaH_2PO_4/Na_2HPO_4)	-	20 min
Elution	4,6		1 M KSCN	10 min

4. Experimental Results

First experimental results show the high potential which this new concept offers. Compared with magnetic separations in an analytical scale the filtration in the filter nutsche offers the possibility to scale up the process with a factor 1000. Once

established, the scale up can be increased even further. Fig.3 left shows the time flow of one separation cycle.

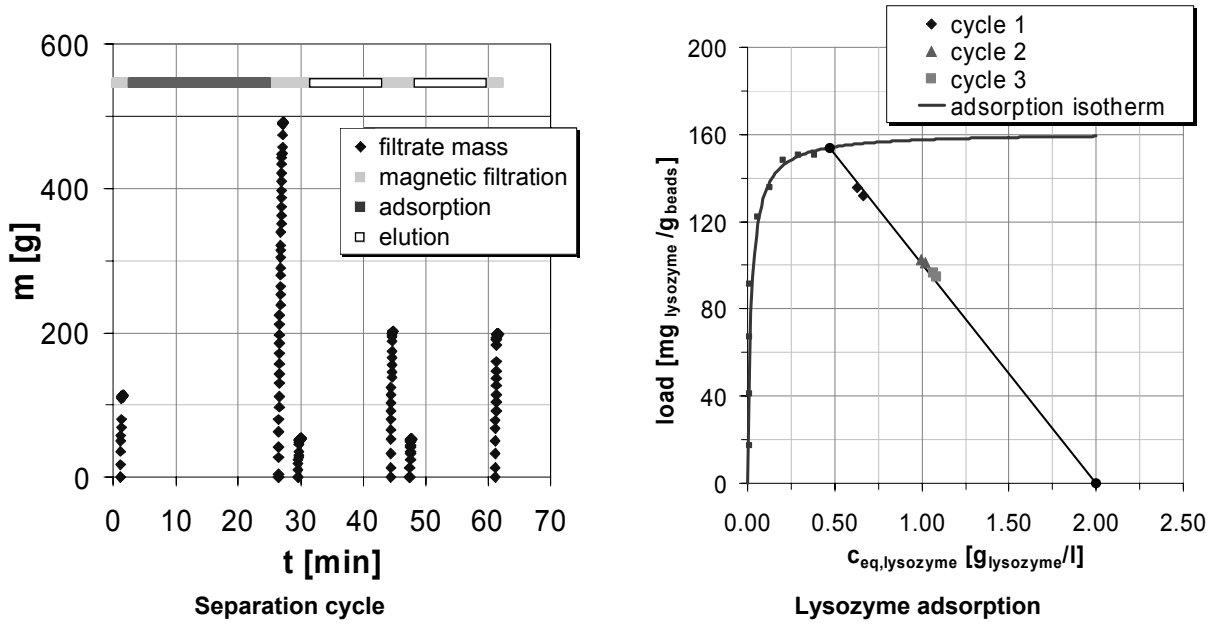

Fig.3: Process steps and adsorption results of the separation process in the filter nutsche

It begins with a pre filtration just to enable comparable starting conditions. Two washing steps with only a small amount of water to flush the particles from the nutsche wall into the inner area of the cell are carried out directly before the elution steps. The whole process is completed within one hour. The time consuming phases are not the filtrations steps but the adsorption and elution times in-between.
The Langmuir adsorption isotherm as the maximum of particle load is measured in a 1ml test tube under ideal conditions (Fig.3 right). All separation data obtained in the nutsche filter is located on an operating line starting at the feed concentration (2g/l). The maximum load of ~87% of the particles in the first cycle is a promising result also compared to other systems. More difficulties causes the elution of the adsorbed enzyme. In the first step only an elution ratio of ~55% is achieved. Thus some lysozyme is attached strongly to the carrier particles so that the pH-shift is not strong enough to detach these molecules. But the variety of applicable chemicals for the elution buffer is limited because the activity of the enzymes still has to be warranted. For the next cycles these positions are masked for adsorption, that way the capacity of the particles decreases. Since in these cycles only the easy elutable regions are charged with new lysozyme, the elution in these cycles reaches values higher than 90%. This point regulates as a constant working point.
Furthermore some of the lysozyme is bound to the surface of the particles due to hydrophobic interactions. Investigations showed the improvement of the elution by adding 1-propanole to the second elution buffer to overcome the hydrophibic interactions at the particle surface.

5. Conclusions

The experiments with the new developed lab-scale magnetic filter nutsche show the potential of the magnetic field enhances filtration in the downstream processing of bio

separation. The separation procedure can be implemented in only one unit operation with a simultaneous up-scale of the factor 1000 compared to the millimeter scale.
The optimal application range is for a product stream with an average concentration of the target product. If the throughput is too small the specific energy costs of the separation unit may be to high, although it has to be considered that by reducing the number of unit-operations an energy saving is achieved. If the target product concentration is too high the effectiveness of the process may suffer from the need of the amount of functionalized magnetic carrier particles, since these still are very cost intensive. Nevertheless in this case also a multi-stage separation procedure with less magnetic beads is possible.
Next step will be the integration in a real bio separation process with several impurity components and a right choice of elution buffers.

7. Acknowledgements

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8. Literature

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