A NEW MAGNETIC FILTER FOR SELECTIVE BIO SEPARATION

Dipl.-Ing. C. Eichholz, Dipl.-Ing. M. Stolarski, Prof. Dr.-Ing. H. Nirschl
Institute of Mechanical Process Engineering and Mechanics (MVM), University of Karlsruhe, 76131 Karlsruhe, Germany; Phone: + 49 721 608 2427, Fax: + 49 721 608 2403

ABSTRACT

One of the emerging fields in the downstream processing of 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 has emerged strongly for analytical purposes. To apply the same concept to industrial bio production processes new technologies have to be provided which allow effective and economical procedures. In this work a new magnetic filter for selective bio separation is introduced. The principle is demonstrated by the recovery of pure lysozyme but it can be extended to real bio suspensions similarly.

KEYWORDS

Bio separation, Cake filtration, Magnetic separation, Magnetic filtration, Adsorption, Lysozyme

1. Introduction

Biotechnological processes use natural metabolisms 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. But biotechnology not only finds its way into pharmaceutical industry, also in the lower value sectors with high throughputs, e.g. food, agriculture, environmental engineering, biotechnology based processes gain more importance (PharmaTEC 2007). However biosuspensions or fermentation broths usually contain side products which have to be removed in time and money consuming purification steps, the so-called downstream processing. It causes up to 80% of the investment and operating costs. Thus especially for the lower value sector new separation concepts have to be found to solve this economic bottleneck. One promising new concept is the so called magnetic separation. By using highly functionalized magnetic substrate particles, which adsorb the desired product like protein, enzyme, DNA etc. selectively, the product can be separated directly out of a mixture of non magnetic components (Safarik, Safarikova 1999; Franzreb et al. 2006). These methods especially have emerged for analytical purposes where only small amounts of functionalized particles are necessary. This is one reason of the lack of process machinery capable for the use within magnetic fields and with the appropriate capacity. To apply the same process concept to industrial bio production processes research focuses on technologies which allow effective and economical procedures.

Investigations mainly focus on the High Gradient Magnetic Separation (HGMS) which uses a magnetic matrix, comparable with a very loose reversible flow deep bed filter, to achieve high field gradients and thus high magnetic forces (Hubbuch et al. 2001). At the University of Karlsruhe also new concepts like the magnetic field enhanced

centrifugation are developed, in which centrifugal forces are applied for discharging a rotating HGMS matrix (Stolarski et al. 2007a). In the present work a new magnetic filter for selective bio separation is introduced which abandons the use of a HGMS matrix. This principle is also known as Open Gradient Magnet Separation (OGMS). Different to HGMS the concept of OGMS is not limited by the capacity of the matrix thus suspensions with higher solid concentrations can be handled.

2. Magnetic separation in the downstream processing

Fig.1 shows the basic principle of magnetic separation. It is independent from the product system and the separation equipment. Only the functionalization of the carrier particles with the ligand coating has to be matched with the actual target product. A wide range of these surface ligands for different target products are already in application, e.g. in diagnostics or in adsorption chromatography. The separation process consists of the following phases: fermentation, mixing, specific adsorption, magnetic separation, washing and elution.

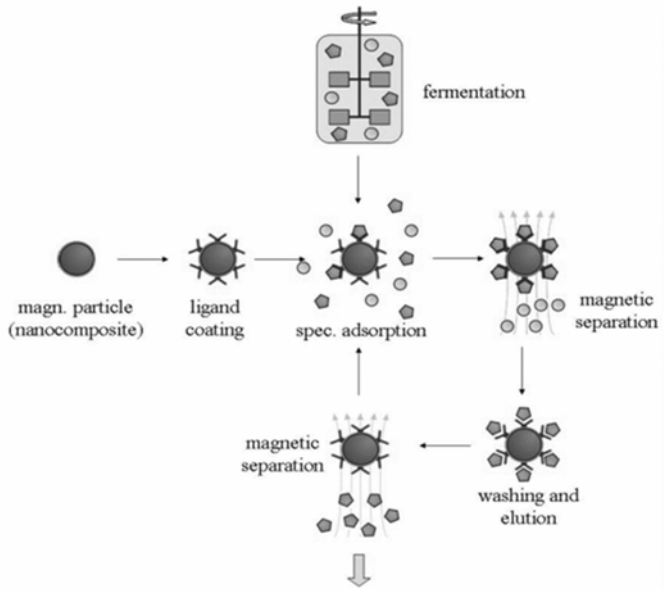

Fig.1: Process schema of the magnetic selective bio separation (Stolarski et al. 2007b)

First the magnetic carrier particles 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 thus the adsorption is not limited by mass transfer. 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. Magnetic field strength and gradient have to be adjusted in a manner that the magnetic force on a particle

$$F_M = V_p \cdot \rho_p \cdot M_p \cdot \nabla B \qquad \textbf{(1)}$$

is dominant compared to the Stokes drag force on a particle due to the liquid flow

$$F_D = 3 \cdot \pi \cdot \eta_l \cdot d_p \cdot u \qquad \textbf{(2).}$$

After the first magnetic separation which separates the loaded magnetic beads from the cell debris the particles are washed several times to reach the desired purity.

Therefore usually 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 followed by a final magnetic separation. For economic purpose the carrier particles are supposed for reusing in multiple cycles.

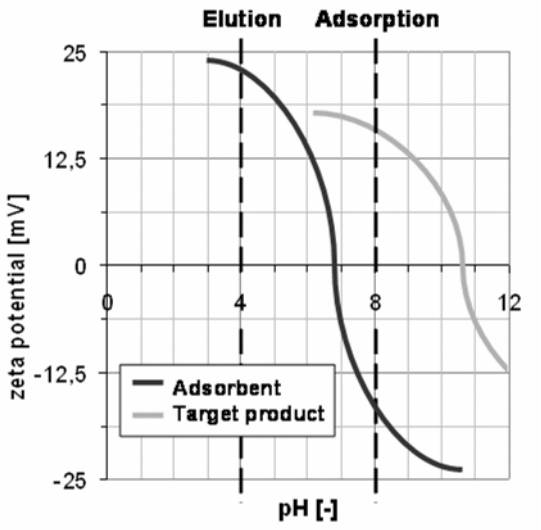

Fig.2: Zeta potential of adsorbent and target product (scheme)

The adsorption and elution mechanisms mainly are governed by electrostatic or hydrophilic and hydrophobic interactions between the bio molecules and the surface of the carrier particles. For adsorption the interactions must result in an attractive force. Selective adsorption is achieved by choosing ligands for the surface functionalization of the carrier particles which – at the right environmental conditions – only induce these attractive forces onto the target bio molecule while the side components are not trapped. For electrostatic adsorption the zeta potential and its devolution with changing pH are essential. Fig.2 shows the zeta potentials of adsorbent and magnetic beads similar to the components used in this work. For adsorption the pH is adjusted in a way that both components have opposed surface charges. By changing the pH-value and hence the zeta potential of one component repulsive interaction forces occur and the molecules detach from the surface of the carrier particles. Often the replacement is supported by adding salt to provide free ions that displace the bio molecules. Simultaneously the salt supports the regeneration of the magnetic beads which is crucial for the efficient use of these particles in a next cycle and their overall lifetime. In the same way hydrophilic/ -phobic effects depending on the surface chemistry of the materials can contribute to attractive or repulsive interaction forces. The overall adsorption behavior can be described by a Langmuir adsorption isotherm.

$$q(c^*) = \frac{q_{\max} \cdot c^*}{k + c^*} \quad \left[\frac{mg_{protein}}{g_{adsorbent}} \right] \qquad (3)$$

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. Especially the latter means an improvement of the downstream processing because every additional unit operation causes further expenses and product loss processing and conveying the biosuspension. Thus the specific costs are reduced by cutting down the expenses and increasing the overall yield. Research is focusing on the development of process machinery capable for the use within magnetic fields and with the appropriate capacity as well as on the improvement of the so called magnetic beads.

3. Methods and Materials

In this paper the process is realized in a magnetic filter. This integrated process allows the combination of all steps in only one unit operation. Fig.3 shows the scheme and a photo of the experimental apparatus. A non magnetic nutsche filter with a filtration area of 50cm^2 is immerged in the bore of a solenoid. The filter media has an average pore size of 15µm. In the experiments a filtration pressure of 0.8bar is applied. The electro magnet has a maximal field strength of 0.7T. The filter cell is positioned in 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 (compare eg.(1) and (2)) which enables the prevention of a filter cake built-up for high field strengths thus the filtration resistance can nearly be reduced to zero (Eichholz et al. 2008). 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 from the filter media. A stirrer is integrated to provide redispersion of the deposited magnetic particles. This is supported by a wash nozzle which allows removing of the particles of the inner wall of the nutsche and which is needed to insert the different buffer solutions. The filtrate flow is registered on a scale. Before the adsorption and elution steps the particles are flushed from the nutsche wall into the inner area of the cell with the help of washing buffer. At the same time residua of the previous steps are removed.

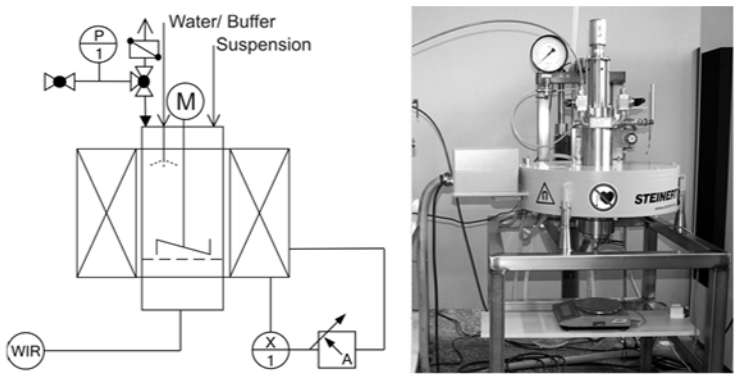

Fig. 3: Scheme and photo of the experimental setup

For all experiments 5g magnetite seeded polyvinyl acetate particles are used with a cationic ion exchanger surface functionalization (Chloroethylammonium chloride) (Franzreb et al. 2007). The beads do not have a remanent magnetization and thus do not agglomerate after turning off the magnetic field. This has advantage in adsorption and elution steps because at all times the whole surface area is accessible as well as

in the reusability of the particles in further cycles. As a model target product lysozyme is used without further side components. To proof reproducibility of the experiments and reusability of the carrier particles in several cycles initial concentration of the lysozyme suspension always is 2 g/l. The exact properties of the magnetic beads and the lysozyme are listed in Tab.1. The lysozyme concentration in the liquid phase is measured with a photometer. The lysozyme load of the magnetic beads is calculated out of the measured equilibrium concentrations with an overall mass balance.

Tab.1: Product properties

	Magnetic Beads	***Lysozyme***
Particle Size [µm]	4.49	0.01
Density [g/cm³]	1.47	
Magnetization M_S [Am²/kg]	22.56	-
pH (IEP)	~6.5	~10
Feed concentration [g/l]	10	2

For the different adsorption and elution steps the pH-value is adjusted exactly to induce attractive or repulsive interactions. Due to the nature of the functionalized polymer surface in the present case none of the previously mentioned adsorption mechanisms is dominant. Both electrostatic and hydrophobic interactions play an important role. Therefore two elution buffers are used, as can be seen in Tab.2.

Tab.2: Buffer Solutions

	pH	**Buffer**	**addition**	**time to equilibrium**
Adsorption	8	0.2 mmol phosphate buffer (NaH_2PO_4/Na_2HPO_4), citric acid	-	20 min
Elution I	4		1 M KSCN	10 min
Elution II	4		1 M KSCN, 20% 1-propanol	10 min

4. Experimental Results

Fig.4 shows the time flow and mass signals of a typical separation cycle. The time consuming phases are not the filtration steps but the adsorption and elution in between (see also Tab.2). The different amounts of filtrate mass only depend on the amounts added in each step and may vary for other separation tasks. Anyway the overall cycle time is not dominated by the filtration since the filtrate mass accumulates immediately on the scale. The linear devolution of all signals proofs the prevention of a cake build-up by the applied magnetic field.

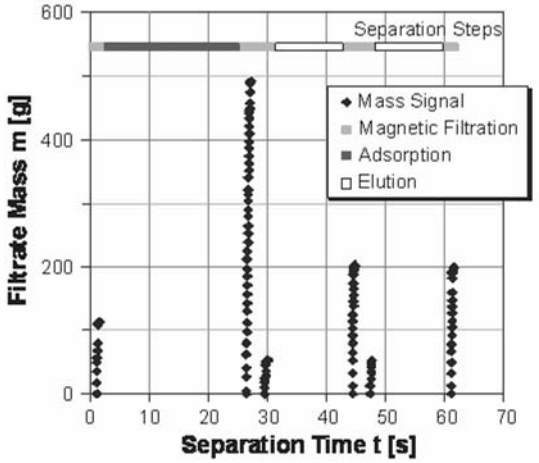

Fig.4: Time flow and mass signals of a separation cycle

The Langmuir adsorption isotherm as the maximum of particle load is measured in a 1ml test tube under ideal conditions (Fig.5). The fit is calculated accordingly to eq.(3). All separation data obtained in the nutsche filter is located on an operating line starting at the feed concentration (2g/l). Due to the attaching enzyme the load of the particles increases while the concentration in the liquid phase drops until equilibrium state is reached. The small gap between the achieved load in the large-scale processes and the isotherm is due to some non idealities in adsorption and measuring inaccuracies. All in all these results show that the whole system is suitable for a multi-step process reusing the magnetic carrier particles.

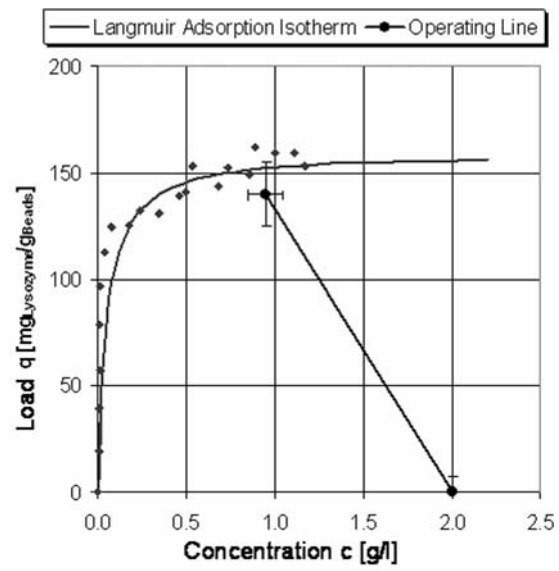

Fig.5: Langmuir adsorption isotherm and operating line

The steepness of the operation line, i.e. the change in enzyme concentration in the suspension, depends on the ratio of lysozyme and carrier particles. To recover as much as possible of the target product two process variations – single and multi stage – can be pursued. In a single stage process the amount of carrier particles must be rather high to provide a large surface area for adsorption which also involves high costs for manufacturing the magnetic beads. Due to the Langmuir adsorption behavior this way the adsorption capacity of the particles cannot be exploited totally. However the overall process time is only a fraction of the term of the multi stage method. The latter is time and buffer solution consuming however less carrier particles are required. The appropriate method must be chosen depending on the particular separation task.

Fig. 6 displays the lysozyme fractions in the adsorption and elution steps. Assuming the solved protein in the feed suspension as 100% round about 60% is adsorbed by the magnetic beads. As aforementioned this strongly depends on the amount of beads used in the process. The remaining protein is flushed out of the system in the magnetic separation step after the adsorption and in the following washing step.

Using both elution buffer solutions high recovery rates of ca. 95% of the adsorbed lysozyme (56% of the lysozyme of the feed suspension) can be achieved. Nevertheless it cannot be removed totally from the particle surface. After a while a constant deposit of some percent is reached and the capacity of the particles decreases. Other additives to the elution buffers may improve even the elution rate but the variety of applicable chemicals is limited because otherwise denaturing of the

proteins may occur. Thus the particles have to be regenerated after several cycles outside the process to ensure a large adsorption surface for the next steps.

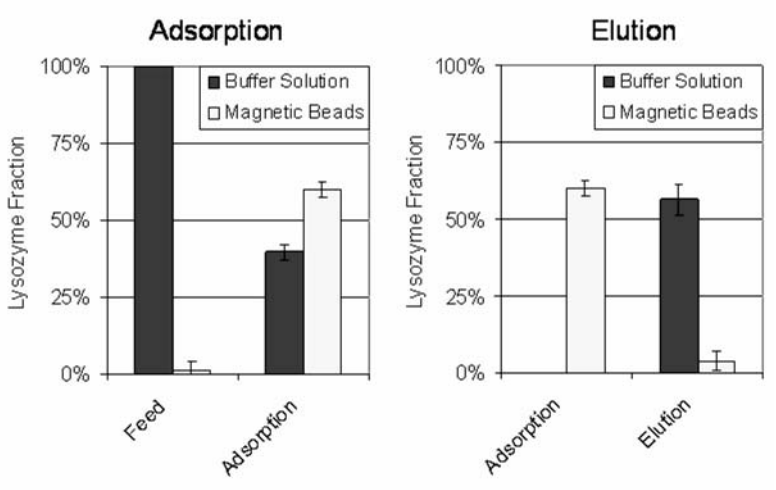

Fig.6: Lysozyme fractions in adsorption and elution

5. Conclusions

The experiments with the new developed lab-scale magnetic filter nutsche show the potential of the magnetic field enhanced filtration in the downstream processing. The separation procedure can be implemented in only one unit operation.

The adsorption and elution of the protein can be transferred into a lab scale application using the magnetic carrier particles in several separation cycles. During the magnetic filtration step the filter media is kept free of particles thus the suspension with all not absorbed components can rinse out of the filter easily. The optimal application range is for average volume flow rates. If the throughput is small the specific energy costs of the separation unit may be too high. If the flow rate is too high a scale-up of the equipment would cause high energy consumption for generating the magnetic field. But anyways not the concentrations of the target product and the magnetic beads are the limiting factors. Next step will be the integration in a real bio separation process with several impurity components.

6. Acknowledgements

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7. Nomenclature

B	T	magnetic flux density
c	g/l	concentration
d	m	diameter
F	N	force
k	g/l	constant

M	$A \cdot m^2/kg$	magnetization
m	kg	mass
q	g/g	load
t	s	time
u	m/s	velocity
V	m^3	volume
η	Pa·s	viscosity
ρ	kg/m^3	density

Indices

D	drag
l	liquid
M	magnetic
max	maximum
p	particle
s	saturation

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