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SELECTIVE MAGNETIC SEPARATION – A REVOLUTION IN SOLID-LIQUID SEPARATION ?

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Abstract
It is well known that the classical methods in solid-liquid separation like filtration and centrifugation approaching their limits when specific compounds from complex particles or molecular mixtures have to be separated. The costs in biotechnology for downstream processes are in general significant higher than the manufacturing costs of a specific target molecule. But the problem to separate a target molecule out of a complex product stream is not just limited to bioprocesses. It is also possible that the target is not a valuable product; also an undesired, specific component has to be eliminated from a product stream. The classical methods have here their limitations because of the small particle sizes of molecular components, the low density difference for biological products and the big amount of similar targets in a complex mixture. In industry usually chromatography, precipitation, crystallisation or membranes are used to separate and to purify the specific targets. Very often one of these methods is not enough to lead to a satisfactory purification and therefore combinations of different types of methods can by found in industry to solve this separation task. Besides with several purification steps a product loss can not be prevented which leads to a low yield and high costs.
Here in the last years the magnetic separation technology made some steps forward into industrial application. Yet the big step into an industrial surrounding is missing because at first a process technology for a magnetic selective separation is still not available. Second, the manufacturing of the magnetic particles with a stable surface functionalization for industrial applications and the reuse of the particles is not clear.
The talk will give an overview of the magnetic separation technology, starting from the particle synthesis, the functionalization until to the process equipment. In the last years some new equipment has been developed and tested for the use in an industrial surrounding. The idea was to use the classical tools of the solid-liquid separation technologies, which are well established and give them a magnetic component to perform a magnetic separation.

Introduction

The basic principle of modern biotechnological processes is the usage 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. 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. However bio suspensions 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 targets like protein, enzyme, DNA, impurities etc. selectively, it can be separated directly out of a mixture of non magnetic components. Similarly e.g. in food industry, the separation of contaminants is conceivable, whereas the target components remain in the main process stream. A wide range of these surface ligands for different target products are already in application, e.g. in diagnostics or in adsorption chromatography. These methods have emerged for analytical purposes already where only small amounts of functionalized particles are necessary.

To apply the same process concept to industrial bio production processes new technologies have to be provided which allow effective, reliable and economical magnetic carrier particle production as well as process machinery capable for the use within magnetic fields and with the appropriate capacity. The biggest challenge is currently the supply of the magnetic beads. Subject of current research are mainly low-cost synthesis and functionalization methods on an industrial scale. Thereby long-term stability and complete elution of adsorpted molecules are of major interest to use the carrier particles in several separation cycles. Besides also the development of effective separation techniques play a non-negligible role. Based on the classic high-gradient magnet separation (HGMS) (Svoboda 2004), where magnetic particles are trapped in a magnetized wire matrix (e.g. steel wool) – discontinuously and only for low concentrations of carrier particles – new techniques without the mentioned disadvantages are developed. In the magnetic field enhanced centrifugation the centrifugal field is used to clean the wire matrix continuously, thus creating a continuous HGMS device. Based on the principle of open gradient magnetic separation (OGMS), where the magnetic particles are separated due to the natural gradient of the magnetic field, an effective filtration process for high particle concentration was developed (Eichholz et al. 2008b).

Magnetic Separation in 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. The separation process consists of the following phases: fermentation, mixing, specific adsorption, magnetic separation, washing and elution. First the magnetic carrier particles, reusable in several separation cycles, are produced and functionalized in a separate process. Then the product-specific functionalized particles are mixed with the fermentation or bio broth. 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. 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 retrieve a pure solution of the final product, it is detached from the particle surface in an elution step. The contaminated washing liquor and the elution buffer are

removed by other magnetic separation steps. For economic purpose the carrier particles are supposed for reusing in multiple cycles.

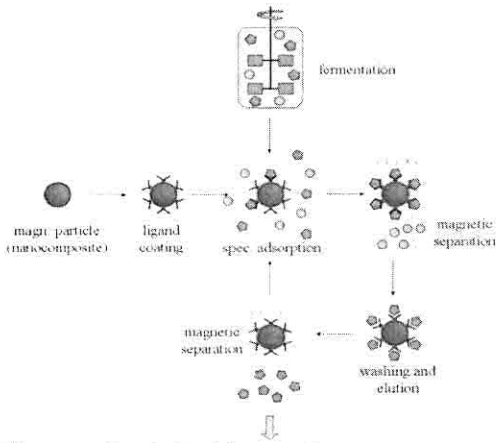


Fig.1: Process schema of the magnetic selective bio separation

The application of external magnetic fields in the separation process has two major beneficial effects to a magnetic particle system. The first one is an external magnetic force that induces a large-scale particle motion. The second effect is an interparticle magnetic potential that results in a microscopic and macroscopic structuring, aggregation and (self) assembly of magnetic particles. The magnetic separation step can be realized in different ways or with different separation devices as will be explained later. 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.

Functionalized Magnetic Beads

As with adsorption chromatography, the carrier particles provide functional surface groups, which are especially suited to the chemistry of the target product (Lattuada, Hatton 2007). Additionally, the carrier particle contains superparamagnetic ferrous components that equip the polymeric base particle with certain magnetic properties. By controlling the magnetic particle concentration the saturation magnetisation is adjustable and can be fitted to the actual process and process equipment requirements. It is crucial that the size of the magnetite particles does not exceed some nanometres, as the particle size determines the magnetic properties, for example the superparamagnetic character of the final magnetic carrier. Superparamagnetism is a phenomenon observable in particles smaller than 10-30 nm, because the particle consists of only one magnetic domain. A domain is a region in which all magnetic spins are co-directed. Additionally, the thermal motion of the atoms has to be strong enough to statistically distribute the magnetic moments after the shutdown of the external magnetic field. Only then the particles can be fully redispersed after magnetisation. This has an 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.

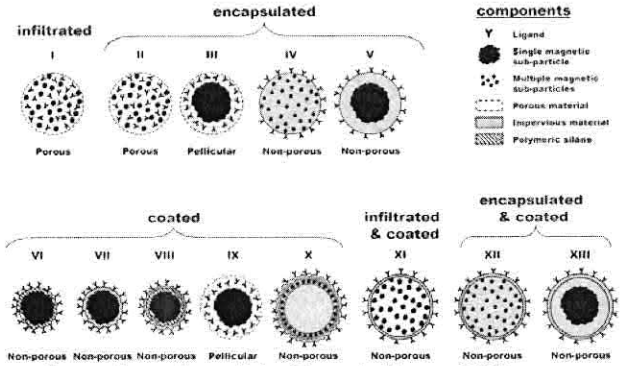
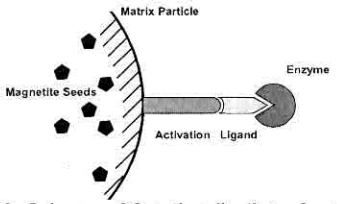


Fig.2: Different types of magnetic carrier particles (Franzreb, Thomas 2009)

Broadly speaking, three different preparation routines or types of magnetic carrier particles exist (Fig.2). Infiltrated particles are prepared by a precipitation of superparamagnetic magnetite from iron salts is initiated inside the polymer matrix. This way a homogeneous distribution of the magnetic component is achieved but the matrix particle must consist of a porous structure. Encapsulated particles exist of a polymer shell with superparamagnetic magnetite incorporated. Possible methods for preparation are emulsion or suspension polymerization. The surface functionalization can be added together with the magnetic seeds or in an additional reaction. Also the direct coating of magnetite seeds, e.g. with an amine layer, is persued and has advantages for the preparation of extremely small particles. Also combinations of the different routines are possible, e.g. infiltrated and coated, if the inner porosity of the particles should be sealed or mechanical or thermal stability of the particles should be improved.
The smaller the particle size, the higher the specific surface area and therefore the capacity of the carrier particle to bind target products to its surface. Functionalized particles between 1 µm and 2 µm typically provide capacities of 150–200 mg/g of carrier particle, while nano-scale magnetic adsorbents bind above 500 mg/g. On the other hand the particles get harder to handle in the whole process the smaller they are. Depending on the separation device an optimum has to be found.



Ligand	Target
cation exchanger	molecules with negative net charge
anion exchanger	molecules with positive net charge
Streptavidin	biotinylated proteins
Protein A, Protein G	monoclonal antibodies
metal chelate	his-tag-proteins

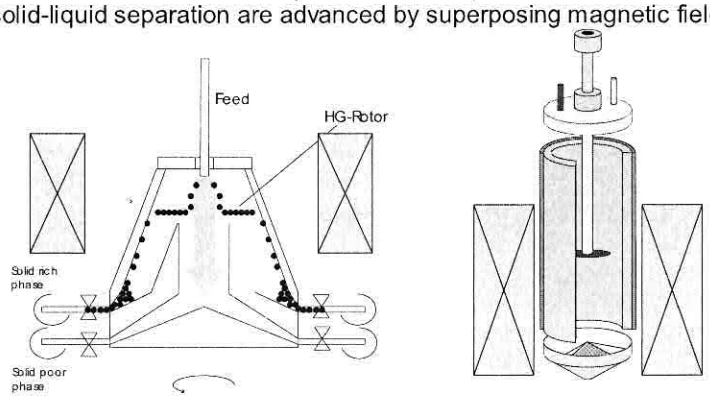
Fig.3: Schema of functionalization of a magnetic bead surface (left), examples of target molecules and corresponding ligands (Käppler 2009) (right)

The ligands for the particle functionalization can be attached to the particles in a separate preparation step or during the particle syntheses. The first case has the advantage that for different applications similar basic particles can be used. Thus a modular design of the magnetic beads could be established (Fig.3). The literature already gives vast options of ligands which can be chosen for the different target products (e.g. (Franzreb et al. 2006)). But up to now, realizing this surface treatment in large scale often incurs high costs and is considered the bottleneck of the industrial

scale carrier production. Intense research work in this sector aims to minimise these costs.
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 conditions – only induce these attractive forces onto the target bio molecule while the side components are not trapped. The elution is realized by a change of pH, ionic strength, temperature or similar, depending on product and binding properties. A complete removing of all adsorbed proteins is tended in the elution step because this is crucial for the efficient use of these particles in a next cycle and their overall lifetime.

Magnetic Separation Equipment

Up to now mainly High Gradient Magnetic Separation (HGMS) filter devices which consist of a magnetic matrix, comparable with a very loose reversible flow deep bed filter, are applied in selective magnetic separation. By introducing the matrix into a preferably homogeneous magnetic field the external field is deformed. From the deformation a high gradient in the direct neighbourhood of the wires results and thus high magnetic forces are generated towards the wires. Even though the deformation is locally concentrated, a closed high gradient magnetic field can be generated by carefully chosen wire diameter and distance. The disadvantages of HGMS setups are the batch wise operation at a fairly low matrix capacity. At the University of Karlsruhe new concepts like the magnetic field enhanced centrifugation and magnetic field enhanced cake filtration are developed. Therefore processes and machinery from classical solid-liquid separation are advanced by superposing magnetic fields.



a: Continuous High Gradient Magnetic Separator b: Magnetic Nutsche Filter

Fig.4: Magnetic Separation Equipment developed at the University of Karlsruhe

The magnetic field enhanced centrifuge is composed of a centrifuge which is installed in the bore of a solenoid (Fig. 4a). As in conventional HGMS a rotating magnetisable matrix, which is positioned in the centre of the centrifuge, is used to collect the magnetic components out of the feed suspension. The superposed centrifugal forces are applied for discharging the rotating matrix continuously. By regulating the magnetic field strength, the g-factor and the differential rotation speed between centrifuge and matrix the process can be adjusted to the actual separation task and properties of the magnetic carrier particles. Finally the realization of a continuously working separation device combined with an effective discharge of the particles from

the matrix wires is realized. In contrary to conventional HGMS, the newly developed machine allows a continuous feeding and discharging of all flows (Stolarski et al. 2008). Compared with HGMS the magnetic field enhanced cake filtration abandons the use of a HGMS matrix. This principle is also known as Open Gradient Magnet Separation (OGMS). It features a higher cleanability of the nutsche filter (Fig. 4b) which is of importance regarding hygienic design aspects. Also suspensions with higher solid concentrations can be handled. Advantageously is especially the merging of a whole process (Fig.1), in only one apparatus. Thereby the superposition of a magnetic field leads to a reduction of the overall filtration resistance or the prevention of a cake build-up (Eichholz et al. 2009).

Applications of Selective Magnetic Separation

Below different examples of selective magnetic separation are presented. In the first case two inorganic components (magnetite (d_{50}=15µm) and quartz (d_{50}=2µm)) are separated in the magnetic nutsche filter. The influence of the magnetic field can be seen easily in Fig.5 left. Without magnetic field the magnetite accumulates as the lower layer on the filter media while the quartz is deposited above. By superposing a magnetic field this order is inversed. Choosing the appropriate pore size of the filter media the two components can be separated nearly completely in a multistage process (Fig.5. right). In the same manner in a bio separation step side products like cell fragments can be removed from the feed stream.

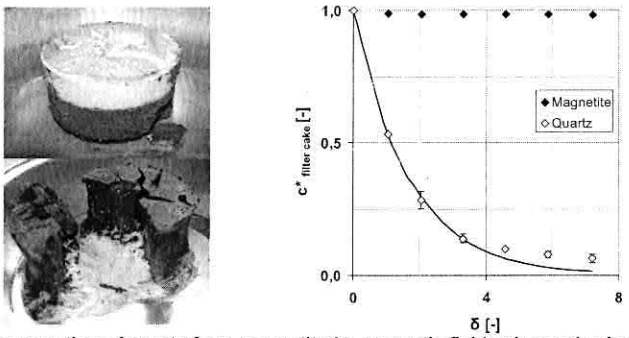


Fig.5: Selective separation of quartz from magnetite by magnetic field enhanced cake filtration: influence of the magnetic field (left), normalized solid volume fractions in the filter cake (right) (Eichholz et al. 2008a)

In Fig.6 the SDS-page of a gel electrophoresis shows the results of separation of lysozyme from hen egg white (HEW) in the magnetic nutsche filter. Therefore magnetic polymer beads with cation exchanger ligands are added to the HEW-solution and separated after the adsorption. Afterwards several washing and elution steps are performed. The bar at the bottom of the page indicates the target protein lysozyme while the other bars indicate other proteins of the HEW solution, mainly ovalbumin. It can be seen that after the adsorption all lysozyme is removed from the liquid phase, which means that it is adsorbed totally on the particles. For elution different steps are performed with only little amount of elution liquor to obtain a high concentrated lysozyme solution. As can be seen the elution is not 100% selective since also other proteins are recovered from the particle surface in the first elution. To retrieve pure lysozyme the functionalization could be improved or the first elution should be discarded. Nevertheless in total more than 95% of the initial lysozyme is recovered with a coincidental improvement of purity from ~3.5% to ~85%.

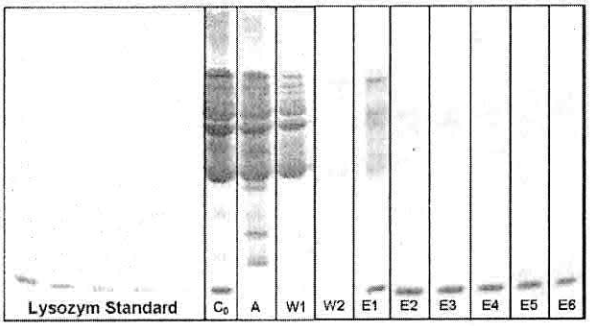


Fig.6: SDS-Page of selective separation of lysozyme from HEW by magnetic field enhanced cake filtration

The last example demonstrates the results of an „In-situ Magnetic Separation (ISMS)" of protease (subtilisin) from fermentation broth (b. lichinifomis) by magnetic field enhanced centrifugation. The in-situ separation of production inhibiting components from the fermentation broth allows the enhancement of the production rate of b. lichiniformis (Käppler 2009).

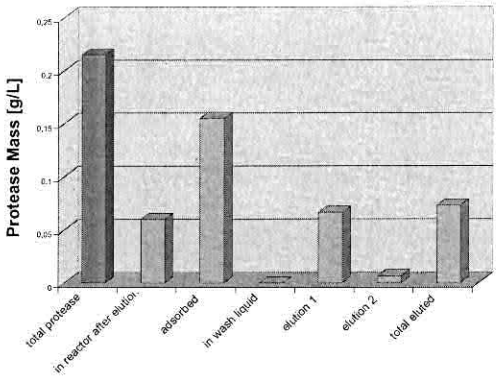


Fig.7: Resulting protein concentrations of selective separation of protease (subtilisin) from fermentation broth (b. lichiniformis) by magnetic field enhanced centrifugation

The progression of the protease concentration over the different process steps is shown in Fig.7. The total amount of protease before the adsorption onto the magnetic particle is approx. 0.21g/l. After adsorption, the concentration of protease in the fermentation broth amounts 0.06g/l. A washing step of the loaded magnetic particles does not involve any loses in the target product, only side products are removed. The first elution results in an amount of 0.07 g/l protease. A second elution step allows further recovery of 0.01g/l protease. A summation reveals that in total only 30% of the produced protease is regained, though in spite of this result 75% of the protease is separated in-situ from the fermentation broth. By an increase of the amount of magnetic beads also a complete adsorption could be realized. Especially the elution behaviour has to be optimized in further works. Either a change of the physic-chemical properties of the elution buffer is possible or other surface ligands should be chosen as functionalization of the magnetic carrier particles.

Conclusions & Outlook

Considering the high costs providing functionalized magnetic carrier particles in industrial scale, further research needs for improving the adsorption behaviour and separation equipment on the one hand and the promising results in selective separation experiments by magnetic field enhanced centrifugation and cake filtration on the other hand the selective magnetic separation will probably not lead to a revolution but can be the right way for some innovative applications and products. Especially when magnetic separation can achieve a considerable volume reduction of the feed stream and save several purification steps it will be competitive with existing procedural paths. To leverage selective magnetic separation to industrial scale is the purpose of a recently started trans-European integrated research project (IP), funded by the European Union – FP7.

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