Continuous Selective High Gradient Magnetic Bio Separation Using Novel Rotating Matrix Centrifugation

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ABSTRACT

Biotechnological production processes using genetically modified bacteria have established as a standard method. As a result of intensive and expensive preconditioning of the fermentation broth product yield is dropping heavily in each processing step and price even skyrockets for some products. Therefore classical downstream processing is considered to be a bottleneck. By utilizing magnetic carrier particles with specific surface treatments a selective separation due to specific interactions of the target product out of the gross bio broth is possible. This approach allows highly efficient product recovery at a minimum of process steps. However this new technology has not been transferred into industrial application yet. Apart from industrial reservation in the utilization of this novel technology not yet existing large scale magnetic separation equipment inhibits the transfer into industrial application. A novel magnetic separator utilizing a rotating magnetic matrix within an external magnetic field is introduced. The combination of the HGMS principle with its high separation efficiency at low particle sizes with continuous centrifugation allows for large scale selective separation. The capacity limitation of classical HGMS filters is avoided by continues cleaning of the magnetic matrix due to centrifugal forces. The proposed paper will present the basics of selective magnetic separation strongly focused on the novel separator design as well as experimental results. The work will be complemented by theoretical considerations.

Keywords: *magnetic separation, bio separation, centrifugation, magnetic field, selective separation, magnetic carrier*

INTRODUCTION

In the last decade recombinant biotechnological production processes have established as a standard method. Due to the high efficiency of the “cell factory” in terms of catalysis and metabolic pathway the production of a wide range of known products as well as even a wider range of new product families has been facilitated. Common fields of applications within the life sciences are situated in agriculture, food science, health and waste water treatment, but are also more and more emerging in the chemical industry.

Typically a fermentation broth or process stream contains not only the desired target product, but also several side products, cell debris and rests of the nutrition media. The extraction of the actual target product is taking place in the downstream process, which consist mainly of separation unit operations like chromatography steps, ultra centrifugation and filtration. Up to 10 different purification steps might be necessary

to recover the product. With 80% most of the final product costs originate from the downstream processing due to its process equipment intensity and high product loss during processing. Even if every separation step works at an efficiency of 95% the total product recovery will drop to 75% in a process chain consisting of 5 and to 60% at 10 steps. In the last years efforts have been undertaken to reduce the number of downstream processes by utilizing hybrid separation technology like magnetic separation [1,2].

The “Magnetic Fishing” (Fig. 1) has been proven to effectively reduce downstream processing effort in analytical applications as DNA separation and protein identification [3]. By utilizing magnetic carrier particles with specific surface treatments a selective separation out of the bio broth is possible due to specific interactions with the target product. These interactions can be natural or artificial by tagging the product. A wide range of these specific interactions has been investigated so far, hence a wide variety of products can be selectively separated. Such products might be certain proteins, enzymes, molecules, viruses, antigens, DNA etc.

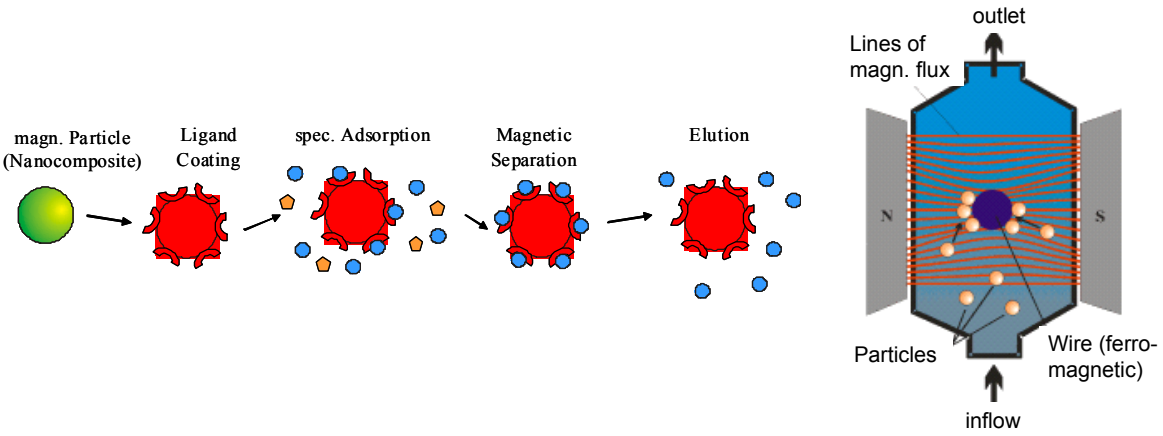

Fig. 1: Process pathway of Selective Magnetic Separation (left), High Gradient Magnetic Separation (right)

The magnetic separation of the product out of broth is based on the magnetic properties of the carrier particles rather than on the actual product properties. Carrier particle properties depend on the manufacturing process and therefore are freely adjustable with respect to particle size, magnetization, surface properties and functionalization. This approach allows highly efficient product recovery at a minimum of process steps. Using batchwise HGMF (High Gradient Magnetic Filtration) assures high separation efficiencies of the loaded magnetic carrier particles at lab scale, but suffers from cleaning insufficiencies and capacity limits at large scale. Consequently this new technology has not been transferred into industrial application yet, even though it is well established in micro scale for analytic applications. Apart from industrial reservation in the exploitation of this novel technology, furthermore high price of reusable carrier particles with proper functionalizations and durability as well as not yet existing large scale magnetic separation equipment inhibit the transfer into industrial application.

Both bottlenecks are worked on intensively in the research community and important milestones could be reached to advance selective magnetic separation in large scale

from vision to reality. One of those is the novel High Gradient Magnetic Field Enhanced Centrifugation.

THEORY

Crucial for the quality of a magnetic separator with respect to size and magnetizability of the particles to be separated is the force that leads to the separation. In case of a magnetic separator the main potential leading to separation is the magnetic force.
According to the general magnetic force eq. 1 high gradient is essential for the separation of fine particulate material as the magnetic force decreases with the particle diameter to the power of 3.

$$F_M = \frac{1}{6}\pi \cdot x_P^3 \cdot \mu_0 \cdot M_P \cdot \nabla H \qquad \text{eq. 1}$$

F_M is the magnetic force, x_P the particle size, μ_0 the vacuum magnetic permeability M_P the particle magnetization and ∇H the external field gradient.
Natural gradients are only of limited magnitude (10-100T/m), therefore HGMF utilizes magnetizable wire grids to artificially distort the background field and create high field gradients. Increasing particle magnetization by higher external field strength is a rather limited option due to the existence of a saturation magnetization.
Depending on the wire diameter field gradients of up to several 1000T/m are generated (Fig. 2). As a result of these high gradients small weak magnetic material can be separated with efficiencies of more than 99%. This enables for the use of micron scaled magnetic adsorbent particles with high adsorption kinetics and high product capacities respectively. That makes high gradient magnetic separation (HGMS) a very powerful tool for separation purposes in selective separation especially under consideration of reusability of adsorbent particles to minimize costs.
Due to the dipolar magnetization of the wires high field regions parallel and low field regions perpendicular to the external field direction are generated.

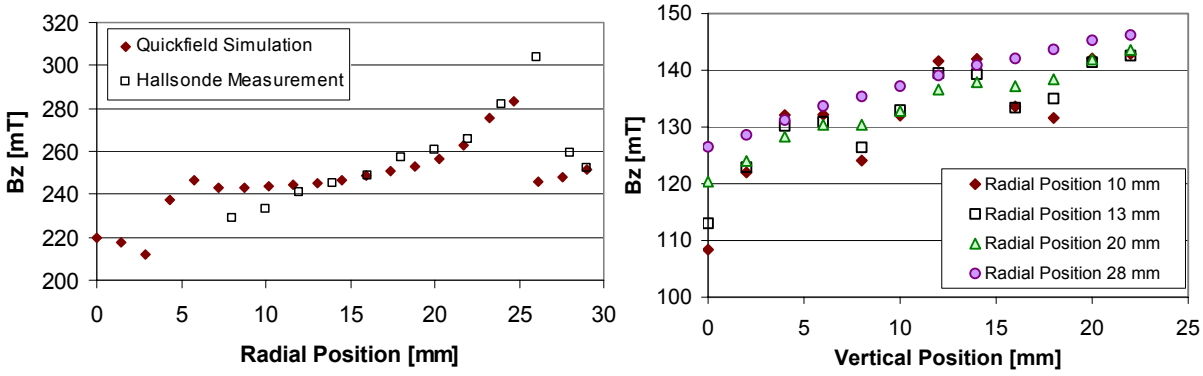

Fig. 2: Vertical component of magnetic flux density at 0.5mm distance along the wire (left), Measurement of vertical component of magnetic flux density between wires as a function of vertical position

Modelling of HGMS separation efficiency is usually based on deep bed filtration simulation incorporating the single wire theory. Herein it is assumed that the wire

grids used consist of assemblies of single infinite wires. Particles approaching the wire in the area spanned by a capture radius R_{Ca} (eq. 2) can be considered as collected on the wire. The capture radius only depends on the axial superficial velocity v_0 and the magnetic velocity v_m which can be calculated according to eq. 3.

$$Rc_a \cong \frac{3}{4}\sqrt{3}\left(\left|\frac{v_m}{v_0}\right|\right)^{\frac{1}{3}}\left[1-\frac{2}{3}\left(\frac{v_m}{v_0}\right)^{-\frac{2}{3}}\right] \qquad \text{eq. 2}$$

$$v_m = \frac{2}{9}\mu_0 \Delta\kappa M_D H_0 \frac{x_P^2}{a\eta} \qquad \text{eq. 3}$$

Δk is the susceptibility difference between particle and fluid, M_D the wire magnetization, H_0 the external field strength, a the wire diameter and η the fluid viscosity.

By calculating the total capture area from the single wire capture radius, which linearly depends on R_{Ca}, the magnetic separation efficiency for one matrix layer can be derived (eq.). However the grids do have a limited capacity, which entails a regular cleaning procedure where the separation chamber is intensively flushed with dispersant liquid to rinse out the captured particles while the magnetic field is powered down. Meanwhile the separation process has to be stopped or conducted by a second process line. An alternative offers the DuPont reciprocating HGMS Filter, where two independent wire matrices are alternately moving in and out of the field of a super conducting magnet system one performing separation while the other one is being washed. [4] Nonetheless the process is far from being continuous.

Thus High Gradient Magnetic Filtration (HGMF) is not suitable for large scale continuous magnetic separation of bio products, as it is a batch wise separation with low capacity of the matrix. Additionally, the matrix materials tend to show fouling at the overlap of warp and weft of the matrix wires. However for the efficient magnetic separation of fine particles a high gradient wire configuration is mandatory, but has to be continuously cleanable during separation.

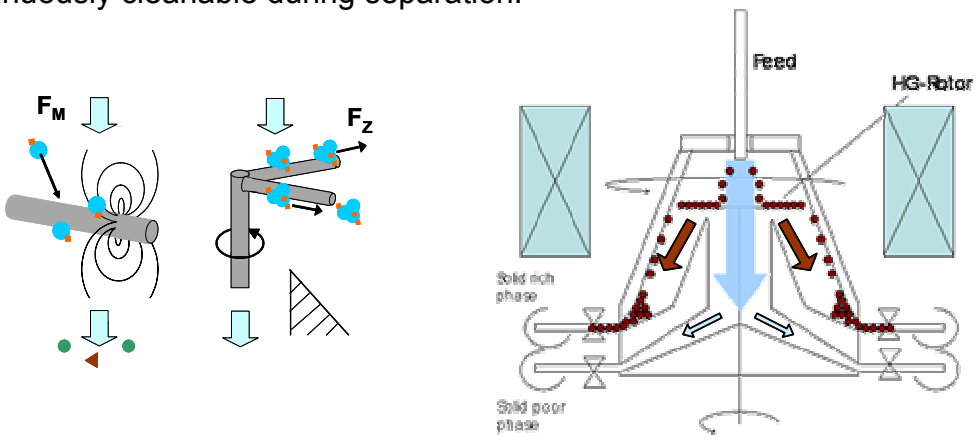

Fig. 3: Principle of Rotating Matrix Centrifuge (DuPont Patent EP000001715956A1)

The transfer of HGMF to a continuous separation process is possible by combining HGMF with classical centrifugation, where the matrix itself rotates in a rotating

beaker. The mixture of bio broth and target product loaded magnetic particles enters the centrifuge. The magnetic particles are attracted by the rotating rod like magnetic matrix and attach to it. Due to centrifugal forces the particles slide along the matrix wires and detach at the end of the wire to be separated into a collection chamber, from where they can be removed either batch wise or continuously (future industrial centrifuge, Fig. 3 right). By adjusting field strength, rpm and differential rpm of magnetic matrix and beaker the new test equipment offers a maximum of control of the separation. The "waste" broth is floating over an orifice and is continuously removed into a non-rotating collector ring.

Three major forces influence the collection, transport and detachment of particles at the rotor: magnetic forces as normal force of the friction force, centrifugal forces and hydrodynamic forces. Due to rigid body motion of the liquid inside the rotating bowl hydrodynamic forces in rotation direction can be neglected while axial flow condition is crucial for the separation. On one hand it determines the residence time of the non-magnetic particles and on the other hand the capture radius of the wire through v_0 in eq. 2.

Influence of hydrodynamic forces on particles already collected on the wire surface shall be negligible at this stage. By keeping the differential rotation of the rotor relative to the outer beaker at zero, the drag force can be minimized or even prevented, which reduces the system to a two force system (Fig. 3 left).

Thereby the forces to be considered during transport of collected particles along the wire reduce to centrifugal force (eq. 4) and friction force with magnetic force as normal component (eq. 5).

$$F_Z = m\,\omega^2 r \qquad \text{eq. 4}$$

$$F_F = \mu_F \cdot F_M \qquad \text{eq. 5}$$

F_Z is the centrifugal force, m the particle mass, ω the angular velocity, r the radius, F_F the friction force and μ_F the friction coefficient.

It is assumed that the particle is captured on top of a stagnant particle layer right above the wire. Here F_M represents the local magnetic force normal to the wire a particle considered to be captured experiences. Field gradient and particle magnetization can be obtained from analytical solutions of the magnetic field around a magnetized single inifinite wire or finite element software for the solution of field problems like Comsol Multiphysics™ or Terra Analysis Quickfield™.

The magnetic forces cause a motion of the magnetic particles towards the magnetized wire, the capturing of particles and an adhesive force of the particles on the wire. The centrifugal forces cause a force component along the magnetized wires towards the wall of the outer rotating beaker and detachment from the rotor.

Additionally to the magnetic separation also the centrifugation will lead to a separation of particles. In calculation this can be considered by forces acting on a particle entering the centrifuge, but neglecting the flow pattern. This approach will be of limited accordance with the measurements as the centrifuge can be considered as an overflow centrifuge, having a main axial flow close to the interface of air core and liquid ring.

However, as experimental results will show centrifugal separation is of minor importance at the operating point of the centrifuge. Therefore the centrifuge can be modeled as a HGMS-filter with rotational cleaning of the matrix.

MATERIALS AND METHODS

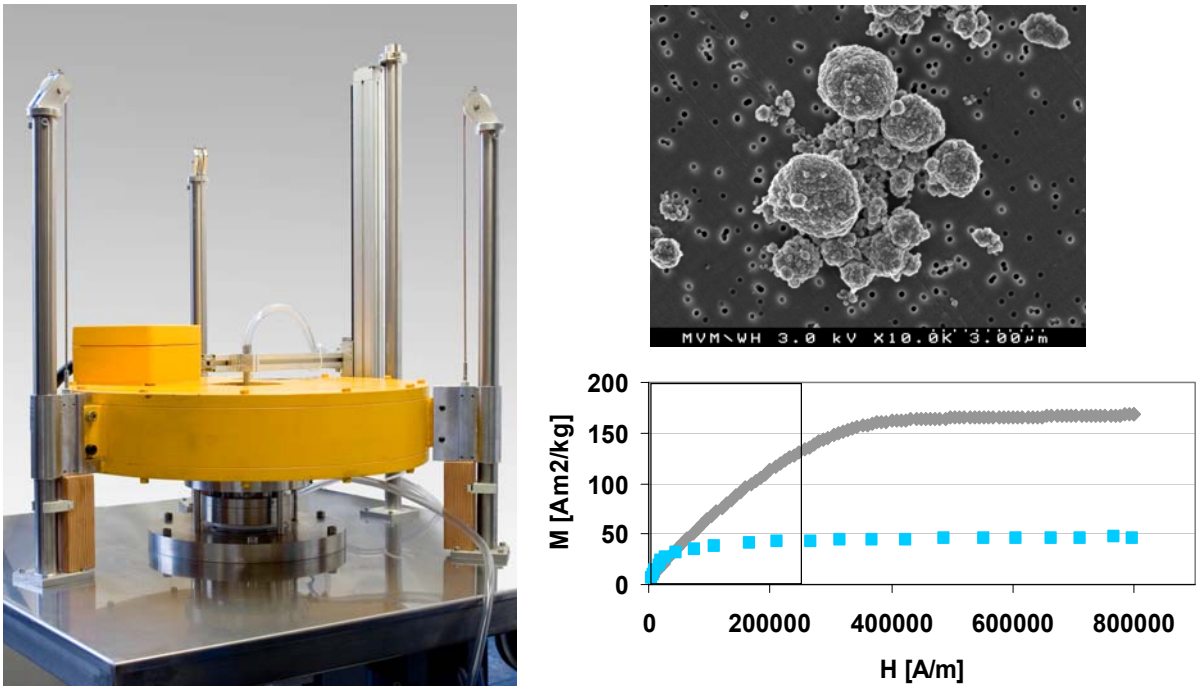

Fig. 4: MEC - Magnetic Field Enhanced Centrifuge (left), SEM picture of Chemagen M-PVA Magnetbeads (top right), M-PVA particle magnetization and 1.4016 matrix wire magnetization (bottom right)

The setup features an electromagnet system with a bore hole of 120mm diameter with a maximum magnetic flux density of 0.4T. The alumina coil is surrounded by an iron frame.
As experimental product for the separation trials a watery 2g/l PVA particle suspension (as shown in Fig. 4 top right) has been prepared and fed into the magnetic field enhanced centrifuge. The saturation magnetization of the superparamagnetic particles is M_s=45 Am²/kg and the mean particles size x_{50}=2µm.
During the experiments the centrate is continuously floating out of the centrifuge and several samples are taken at stationary condition, which has been determined to be after about 3min depending on concentration and rpm.
For the investigation of the separation capabilities of the centrifuge water suspended non-functionalized particles are used. The bio product adsorption has been omitted therefore. Analytics are performed gravimetrically. The separation efficiency is calculated as the ratio of centrate and feed concentration.
For Bio separation experiments Magnetbeads were suspended in fermentation broth of *bacillus licheniformis*, which is an industrially applied micro organism producing

subtilisin. Total bio mass contents of up to 30g/l where cultivated having a viscosity of η=2..4 Pa s. Screening experiments selectively separating subtilisin had been carried out using Bacitracin functionalized Polyglutaraldehyde coated aminosilan particles with M_S=20 Am²/kg and x_{50}=5µm as well as Merck MagSilica 50-85 particles with x_{50}=5..10µm and M_S=43 Am²/kg.

RESULTS

First experiments had been performed using an acrylic rotor lid to enable observation of particle motion inside the centrifuge. By video taping the transport of the particles along the wires and the detachment at the tip of each wire of the rod has been visualized. Conditions after the experiments support the observations. Fig. 5 (left) shows the particle loaded triple layered matrix attached inside the outer rotor. The shape of the attached particles corresponds to expectations showing deposition in line with the external field direction, above and underneath the wire; but no collection perpendicular to the field direction. Particle load increases with increasing radius, due to a radial field gradient. As a result of the detachment of particles the outer wall shows higher particle deposition at each of the three matrix levels. There is not occurring any particle collection at the wire in the centre region as an air core is built inside the centrifuge.

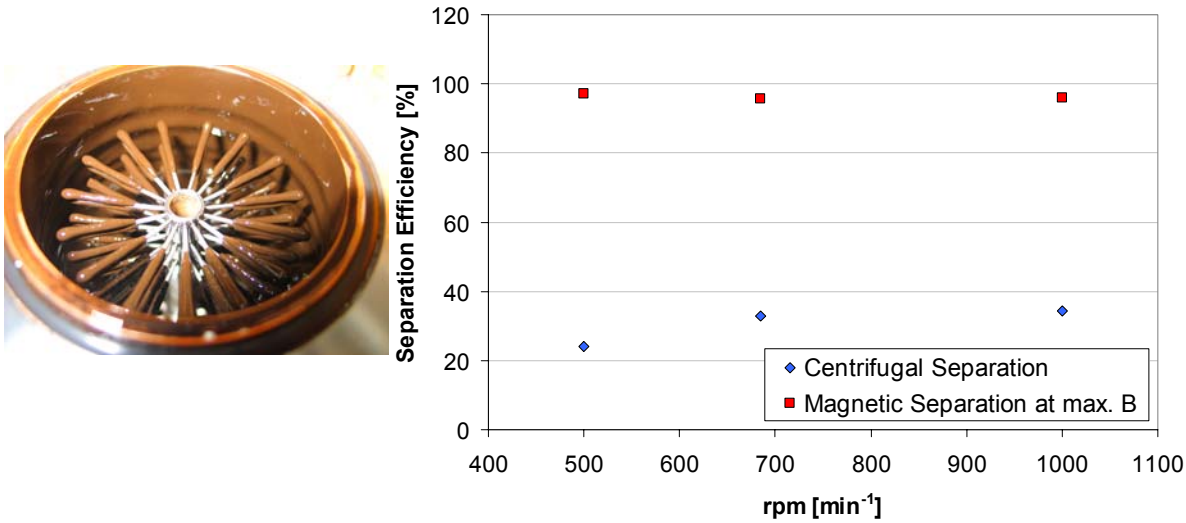

Fig. 5: Particle loaded matrix inside outer rotor (left), Separation efficiency for centrifugal and maximum field magnetic separation (right)

Separation results (Fig. 5 right) without magnetic field are around 24%-34% depending on the c-value. With increasing magnetic field strength separation efficiency rises up to 97% at 0.31T and seems to be independent from the c-value in the investigated region.
For low flux densities the change of separation efficiency is far stronger than at higher ones. Therefore the process shows instability in that region, which is indicated by the higher error bars.

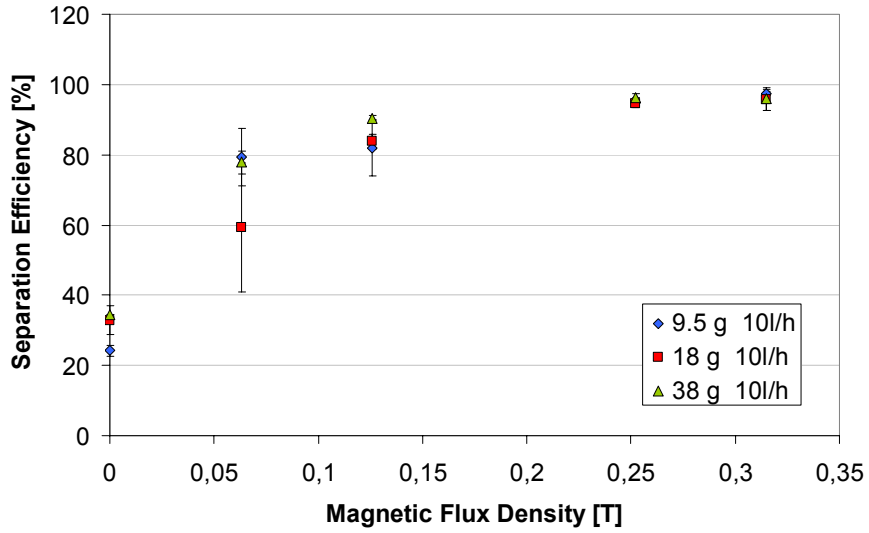

Fig. 6: Separation efficiencies at 10l/h feed stream

Nevertheless in the interesting section of high flux densities - from the separation process point of view - a stable and very efficient separation can be observed. The current setup is a very conservative one with optimisation potential especially in the matrix (wire diameter, number of wires and arrangement of wires) as well as maximum field strength.
Also for flux densities below 0.31T a significant influence of the c-value has not been observed yet, suggesting that theoretical approaches may concentrate on magnetic separation with negligible centrifugal separation at high magnetic flux densities. That means that the overall separation process can be modelled as a magnetic filtration process using single wire capturing with centrifugal removal of collected matter from the wire matrix.
Fig. 7 shows the dependence of the separation efficiency from the feed volume flow. Feed volume flow determines the axial velocity and the residence time of the particles inside the separation chamber. As already mentioned above axial flow velocity influences the single wire capture radius significantly (see eq. 7). For industrial applicability the consequence at high throughputs is a reduction in wire to wire distance. The line shows the reduction of the capture radius with increasing feed volume flow, which can be considered the most influencing parameter to the separation efficiency as percentage reduction as function of feed volume flow is in the same order of magnitude, suggesting that proportionality between efficiency and capture radius should be linear.

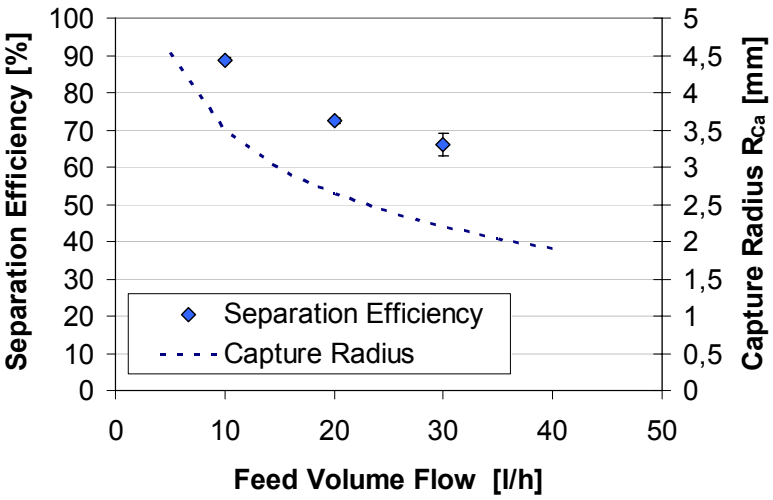

Fig. 7: Dependence of separation efficiency from feed volume flow

Apart from plain separation trials the novel magnetic field enhanced separator was integrated into a fermentation process. Bio separation experiments were carried out with different types of particle systems as introduced in materials and methods. Biological feed stock has been used from *bacillus licheniformis* fermentation broth. *Bacillus licheniformis* had been cultivated to produce proteases. By insitu removal of proteases using bacitracin functionalized PVA particles the production rate of proteases could be increased. The centrifuge successfully separated the magnetic particles with the proteases attached to its surface from the fermentation broth.
Separation was run at maximum flux density of 0.315T (Fig. 8). 75% of Polyglutaraldehyd particles were separated. This fairly low separation efficiency is caused by very weak magnetization MS=20Am²/kg of the particles. Commercially available particles feature saturation magnetizations from 30 Am²/kg up to 45 Am²/kg. Also the bigger particles size does not compensate the lack of magnetizability. However by increasing the number of wires, decreasing the wire diameter of the matrices and increasing the field strength which is in this case limited by the design of the electromagnet efficiency could be increased significantly.
Another exemplary separation from fermentation broth was done with MagSilica 50-85 which has higher saturation magnetization and bigger mean particle size. 100% magnetic particle separation could be achieved.
When processing fermentation broth the apparatus is challenged harder than with watery slurries, because viscosity is usually higher, cell debris and nutrition media is still present. To increase separation selectivity which is a major point in purification of bio products, centrifugal separation should be kept at a minimum as it is unselective. This is realized by keeping the rpm low, but high enough to initiate transport on the wire, the actual separation job is done by the magnetic separation, which is highly selective especially at low magnetic particle concentrations.
During the bio separation trials no measurable bio mass content could be observed in the separated particle fraction, which means selectivity is close to 1.

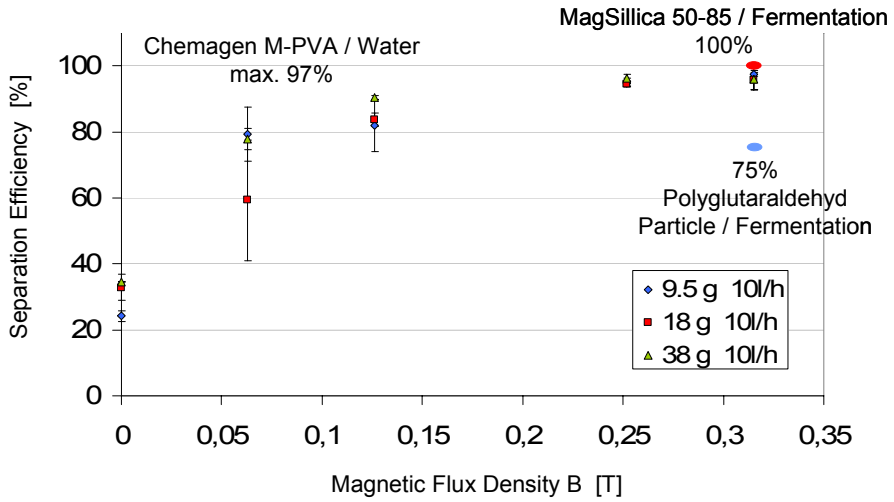

Fig. 8: Magnetic Bead Separation from Fermentation broth of *bacillus licheniformis*

CONCLUSIONS

The new hybrid separation device has proven to be a very efficient and flexible tool for the separation of magnetic particles out of biological feed stocks. By utilising High Gradient Magnetic Wire Matrices it is capable of processing weak magnetic material and/or small particle sizes. Due to its design a continuous separation is possible. The authors see high potential in the area of bio separation using surface functionalized magnetic particles for bio affinity driven selective separation.

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