Chapter 12
An Industrial Approach to High Gradient Magnetic Fishing in the Food Industry

Christian Fiil Nielsen, Karsten Keller, Susanne Rasmussen, Niels Dalgaard, Timothy John Hobley, Wickie Sondergaard, Hermann Nirschl and Johannes Lindner

Abstract High-gradient magnetic filtration bases on the separation of target media by synthetic particles with magnetic core and an adsorbent immobilized on the surface. The process was used in a pilot line at liter-scale for the separation of a protein Bowman-Birk protease Inhibitor (BBI) out of an industrial coproduct stream in the soy industry. The target protein has potential applications as pharmaceutical or food additive. The starting medium is challenging as the product cost is low, its concentration is low and its purity needs to be high. The main method tested was magnetically

C.F. Nielsen (✉) · S. Rasmussen · N. Dalgaard
DuPont Nutritional Biosciences Aps, Edwin Rahrs Vej 38, 8220 Brabrand, Denmark
e-mail: Christian.Fiil.Nielsen@dupont.com

S. Rasmussen
e-mail: Susanne.Rasmussen@dupont.com

N. Dalgaard
e-mail: Niels.Dalgaard@dupont.com

K. Keller
DuPont, Wilmington, DE 19980, USA
e-mail: Karsten.Keller@dupont.com

T.J. Hobley · W. Sondergaard
Technical University of Denmark, Søltofts Plads Building 221,
2800 Kongens Lyngby, Denmark
e-mail: tjho@food.dtu.dk

W. Sondergaard
e-mail: wics@food.dtu.dk

H. Nirschl
Institute of Mechanical Process Engineering and Mechanics, Karlsruhe Institute of Technology, 76131 Karlsruhe, Germany
e-mail: hermann.nirschl@kit.edu

J. Lindner
Karlsruher Institut für Technologie (KIT), Institut fuer Mechanische Verfahrenstechnik und Mechanik, Geb. 30.70, Straße am Forum 8, D-76131 Karlsruhe, Germany
e-mail: johannes.lindner@kit.edu

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enhanced centrifugation in a batch-wise mode with anion exchange ligands. The separation of the target protein at large scale was successful and economical. The efficiency was low due to the low ligand selectivity and the batch-wise processing. For commercialization, high ligand selectivity and continuous processing would make this new magnetic separation process very attractive. An economic study was performed to determine the influence of different parameters on prices. Competing technologies were evaluated. A risk analysis of nanoparticles used in the pilot line was performed.

12.1 Introduction

In the food industry, an increased effort is done on the further use of coproduct streams from the production, partly for sustainability reasons, partly because the disposal costs of aqueous wastes increase. Instead side-streams containing biological material are increasingly transformed into useful products or feed stocks for other processes. A large amount of water is used in the processing of food, leading to very dilute side-streams. More sustainable processing becomes increasingly important, and is hence a goal of the current project. Traditional separation processes are costly, resulting in a need for new approaches.

The current chapter covers the process development and optimization in lab and pilot scale. Considering the target purity and the market price, an ion-exchange ligand-based multistage process was selected. Anion exchange resins were chosen as a consequence of the isoelectric point of the BBI. Substantial pretreatment steps showed to be necessary for the final process due to the low selectivity of ion-exchange functionalization. BBI-specific ligands are technically possible, such as antibodies and phage display of peptides, but for the specific system not available. If available they would be excessively expensive, as discussed below.

Out of the particle systems developed, two systems were produced at a scale sufficient for the pilot line, the Merck MagPrep TMAP, introduced in Chap. 7, and SolPro from Chap. 6. Additionally, commercial particles thankfully provided by Orica Watercare were tested.

Three magnetic separation devices were introduced in part II. The Halbach magnet arrangement from Chap. 8 was tested on the system, the results were presented in the respective chapter. Results on the CME from Chap. 10 on the separation of proteins are presented elsewhere. The most promising separation principle for this project seemed to be magnetically enhanced centrifugation (MEC, US patent 8012357) presented in Chap. 9. It was chosen for its high volume flow up to 1 m^3/h in a 6 L chamber (not counting dead times in the batch-wise machine) and the option for continuous use. As only one machine was available, the process was realized batch-wise. The separation steps adsorption, washing, elution, and another washing step were performed subsequently in one MEC. In a continuous process, four machines would be necessary (two in case of omission of the washing steps). The performance was already shown in Chap. 9.

An important aspect of the evaluation of a new technology is its feasibility in an industrial setting. Target is the evaluation of economic aspects and of the

process safety for operators, environment, and the consumer. Additionally, an overview over the footprint in terms of sustainability is necessary. Finally, the technology needs to be competitive with other technologies applied to the same task, both from a process footprint and economical point of view.

12.2 The Raw System and Pretreatment

One example of a complex food production side-stream containing a desirable product is soy whey from the production of soy protein isolates. The soy whey contains suspended solids and a range of dissolved components, e.g., carbohydrates, salts, and proteins. One of these proteins, Bowman-Birk protease Inhibitor (BBI), has attracted attention lately, most notably due to alleged cancer prevention properties. The BBI in soy whey is difficult to separate by traditional downstream processing as the stream contains only low concentrations but significant amounts of other proteins.

In Table 12.1, important process variables of the food pilot line are presented. Raw material of this process is the non-pretreated soy whey.

The soy whey for all lab and pilot scale experiments was obtained from one of Solae's soy protein isolate plants. To ensure constant sample quality the soy whey went through a rigor pretreatment process. The soy whey from the production line was transported cooled, heat treated using steam, stirred and neutralized from the acid pH of the soy whey by concentrated NaOH. Then 1 % of the silica gel Geduran SI60 from Merck was added and stirred for 15 min to adsorb unwanted proteins. The target protein bound as well to Silica, but to a much lower extent compared to other proteins. The Silica was removed from the suspension, by sedimentation and the centrate and overphase is filtered through 40, 10, and 0.2 μm filters to completely remove silica, suspended solids, and microbial contamination. The soy whey was then preserved with 0.05 % Proclin 300 from Sigma Aldrich.

Table 12.1 Important process variables for the layout of the food pilot line

Process variable	Value
Total protein concentration in the raw material	0.1–1 %
Ionic strength/conductivity of the raw material	5–15 mS/cm
pH value of the raw material	4.5 ± 0.2
Solid content of the raw material	Low
Concentration of other critical components in the raw material	0.5–2.0 % dissolved sugar
Separation relevant target product properties	IP $\approx$ 4.2
Stability of the target product in the raw material	Stable (especially with respect to temperature)
Target product concentration in the raw material	100–500 mg/L
Aspired target purity end product	>90 % (>1,000 units/g)
Aspired market price of the end product	>250 €/kg

Table 12.2 Composition of pretreated soy whey batches

Batch number	Protein via BCA (mg/g)	Ci activity (U/kg)	Specific activity (U/g)	Conductivity (mS/cm)	Sugar (mg/g)	Ash (mg/g)
Raw	2.6	373	146	10.12	11.5	6.8
Heat treated 1	1.74	389	223	9.02	n/a	n/a
Heat treated 2	2.0	243	121	7.90	9.6	5.5
Heat treated 3	1.8	471	265	6.28	9.71	n/a

Four batches of soy whey were produced in different stages of the project, one from raw whey and three from industrially heat treated streams. Table 12.2 shows the composition of soy whey and the batch to batch variation. The differences also affect variation between trials. To eliminate these variations, all trials run in pilot scale, both in the food pilot line and in the academic MEC, CME, and HGMS use the same batch 3, as well as a single batch of Orica MIEX DOC and Magprep TMAP particles.

12.3 Lab Scale Process Development

First operating conditions were investigated in lab scale on different bead system and on parameters like pH, conductivity, and bead concentration. Samples were analyzed by reducing SDS-PAGE, which showed changes in the product composition due to different affinities of each protein species. A pH-value of 7 showed to give good adsorption.

Purification of BBI from pretreated soy whey by adsorption to MagPrep TMAP particles of different concentrations is shown exemplary in Fig. 12.1 left. The proportion of separated product to contaminating proteins showed to be higher at high

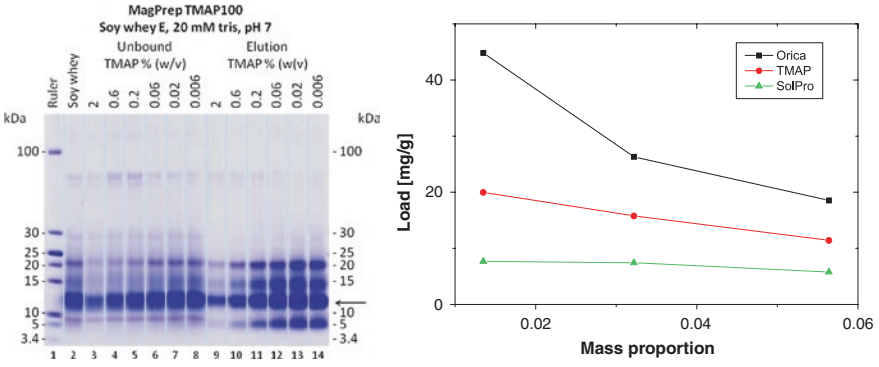

Fig. 12.1 *Left* SDS-PAGE gel of purification in soy whey at pH 7 using different concentrations of TMAP particles; *right* protein binding capacity Q of Orica, TMAP and SolPro adsorbents at three different particle concentrations in pretreated soy whey at pH 7

TMAP particle concentrations of 2 %. Purification of BBI from pretreated soy whey was tested by the anion exchange magnetic particle systems Orica MIEX DOC, SolPro, and Magprep TMAP. Three different particle concentrations (1.5, 3 and 6 %) were examined. The highest binding capacity was achieved at a particle concentration of 1.5 % for all particle systems. While the maximum adsorbed amount of the model protein BSA as a pure substance was 1,150 mg/g, nonprotein contamination reduced the adsorption significantly. Orica and Merck TMAP particles bound up to 22 and 15 mg/g, and SolPro bound 7–8 mg protein/g (see Fig. 12.1 right).

The unbound fractions and the eluded protein were analyzed by SDS-PAGE in Fig. 12.2. The first elution showed to be sufficient for most of the protein, while

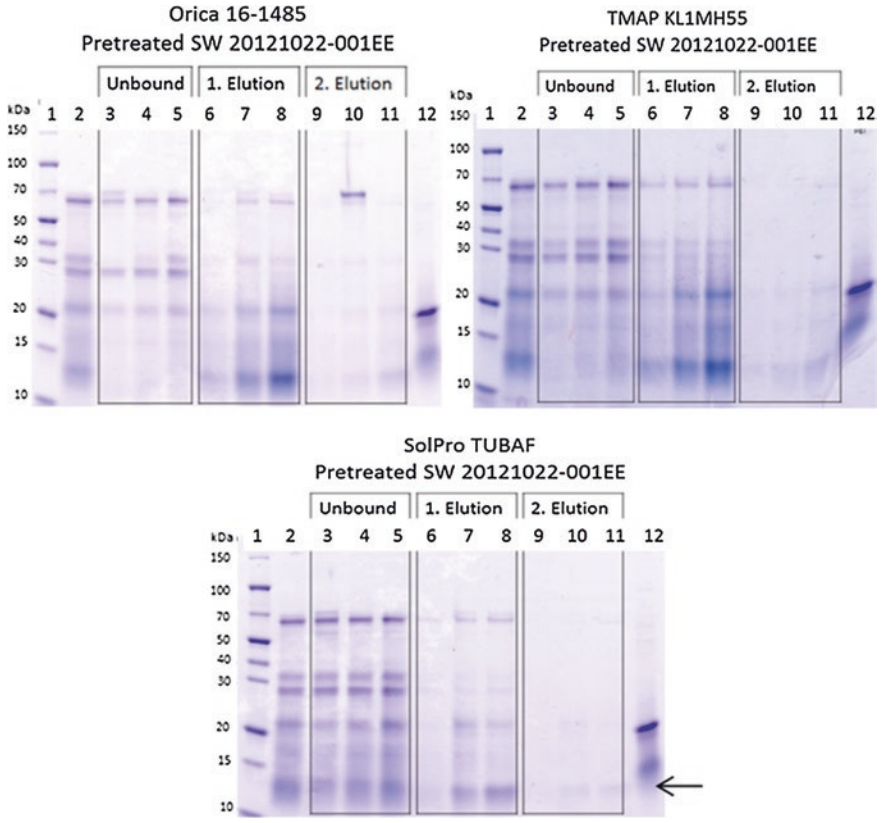

Fig. 12.2 SDS-PAGE gels of purification with the anion exchange magnetic adsorbents (*Orica, TMAP and SolPro*) used for pilot trials at 3 different particle concentrations. Elution was conducted using 0.5 M Na_2SO_4, 20 mM Na_2HPO_4, pH 7 buffer. The *arrow* shows the position of the BBI target; lane *1* molecular weight markers; *2* soy whey before addition of magnetic particles; *3–5* unbound proteins after purification with magnetic particles with *6*, *3* and 1.5 % *Orica*, TMAP or SolPro particles; *6–8* first elution at *6*, *3* and 1.5 % Orica, *TMAP or SolPro particles 9–11* second elution at *6*, *3* and 1.5 % Orica, *TMAP* or *SolPro* particles; *12* 14 µg BBI from Sigma

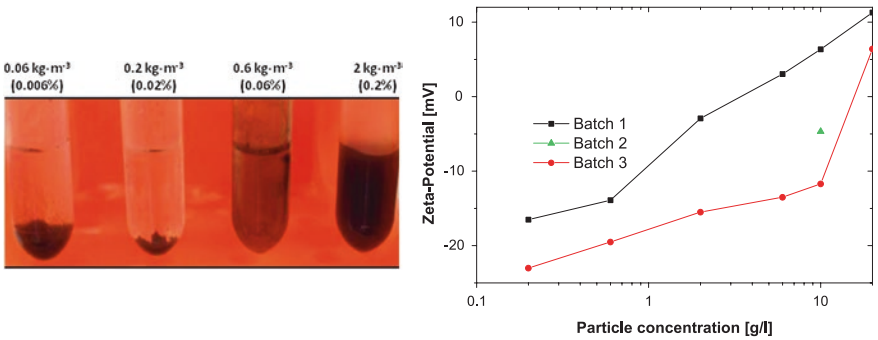

Fig. 12.3 *Left* TMAP particles exhibit different physical behavior at different particle concentrations in soy whey; *right* zeta potential of different Merck TMAP batches at different concentrations in soy whey

a second elution was necessary to elute virtually all of the protein bound to the adsorbents. Due to the low yield, in the final process, 2 % particle concentration was used.

The optimization aimed to replace chemicals not suitable for large-scale processes as well as identifying a process with a minimum consumption, acceptable yield, and robust conditions for upscaling. Magnetic particles were washed after binding to remove unbound contaminant protein. One washing cycle showed to be sufficient for this purpose. Washing was done for 10 min in 1 mL 20 mM Tris-HCl at pH 7.

Figure 12.3 left shows different concentrations of Merck TMAP particles in the same product. The particles precipitated to a highly porous deposit at a particle concentration of 0.006 %, a lowly porous deposit at 0.02 % and formed a colloidal suspension as the particle concentration was increased to 0.06 and 0.2 %. Ionic strength and pH needs hence to be controlled to keep the binding capacity, the porosity, and the separation efficiency in a magnetic separator constant.

The zeta potential of the different TMAP particle charges (Fig. 12.3 right) ranged from slightly negative at 0.006 % in whey to highly positive at concentrations above 0.2 %. TMAP particles showed to be unstable and difficult to resolve from walls at a neutral charge, while an amount of 2 % (20 g/l) of particles could be handled easily. A similar behavior was not identified on different particle systems due to the particle size of nonporous 100 nm Merck particles to 150–180 μm macro-porous Orica particles.

12.4 The Large Scale BBI Production

The system was tested in two approaches. A small batch-wise MEC of 1 L filter chamber volume showed the feasibility of the process. Subsequently separation in a MEC of 6 L volume, produced by Andritz KMPT, was performed to show

the pilot line scale of 1 m^3/h. Both devices and their performance were already presented in Chap. 9, the smaller as well with a permanent magnet.

12.4.1 High Gradient Magnetic Fishing in a Small Pilot Line

The BBI separation was tested in a batch-wise magnetically enhanced centrifuge on the two different particle systems Orica MIEX DOC and Magprep TMAP. In the test, 1.25 L of soy whey were adjusted to pH 9 with NaOH and mixed for 10 min with 25 g of Merck TMAP particles, respectively 2.5 L of soy whey with 50 g of Orica MIEX DOC. 0.7 L of washing water for each washing step was set to 20 mM sodium phosphate buffer at pH 7, and 0.7 L of elution buffer was set to 20 mM Na_2HPO_4 at pH 8 and 1 M sodium chloride. After 20 min adsorption, the suspension was fed in the machine. The magnetic particle separation was performed at 1,500 rpm, 0.2 T and 40 l/h. The centrifuge was drained with magnetic particles left in the machine at applied magnetic field without centrifugal velocity. To remove the remaining particles in the centrifuge, the centrifuge and magnet was switched off and washing liquid was fed in the machine. To resuspend, 3 min washing with stirring at 180 rpm was performed. After 5 min sedimentation at 600 rpm and 0.2 T, the machine was drained. This step was repeated with elution buffer and washing buffer. At the end of the run, the machine was discharged. The result for the protein content in each process step is shown in Fig. 12.4, with Orica MIEX DOC particles (left) and Merck TMAP particles (right). Both particle systems separated effectively BBI. The process was hence effectively transferred from the lab scale to magnetically enhanced centrifugation without large deviation in the separation, as results from lab scale are similar. The scale was only limited by the available amount of magnetic particles.

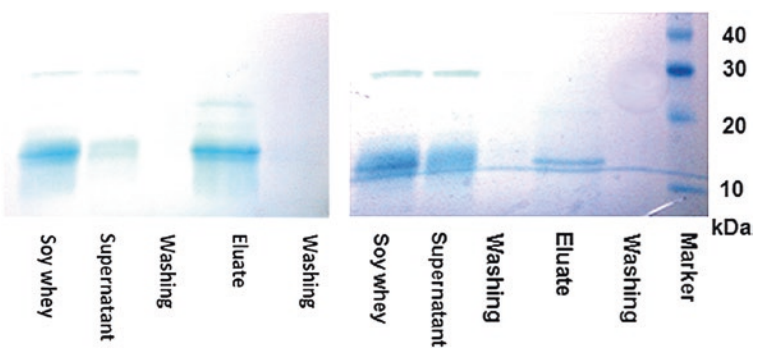

Fig. 12.4 *Left* SDS-Page gel for Orica MIEX DOC particles shows selective separation with the last contamination disappearing, a large part of the BBI is adsorbed and a part eluted; *right* TMAP shows as well adsorption and desorption of BBI

12.4.2 High Gradient Magnetic Fishing in a Large Pilot Line

For the final scale of the process, the pilot line was based on the large centrifuge of 6 L filter cell volume with 20 wire stages and a water cooled electromagnet of 400 mT flux density. Chemicals were changed to transfer the process to a pilot line. Tris-HCl was used at lab scale but cannot be used in the food industry. It was therefore replaced by 20 mM NaH_2PO_4. Similarly, 1 M NaCl was used for elution, which causes corrosion on the equipment. It was replaced by 0.5 M Na_2SO_4. The change in buffer and elution did not have a significant impact on the purification results.

For an exchange of the liquids, a displacement was performed from top to bottom. The elution liquid was displaced bottom to top to avoid mixing of the less dense salt suspension. The resuspension was done at a volume flow of 240 l/h, while stirring in both directions with the matrix for 5 min. A valve system was used to feed the centrifuge forward or backward. An amount of 300 g particles were used to process 15 L of soy whey, corresponding to a concentration of 20 g/l. Before starting the process, the particles were trapped in the centrifuge, washed, eluded, and washed again.

- First the adsorption was done. For this purpose, the magnetic field was applied and the liquid in the centrifuge displaced with soy whey from bottom to top at a volume flow of 60 l/h. Then the liquid was pumped at 240 l/h in a cycle for 5 min, while the matrix was used as a stirrer at 120 rpm to redisperse the magnetic particles. No centrifugal velocity was applied in this step.
- The washing liquid in the centrifuge was replaced by soy whey and the particles were redispersed for 5 min similar to step 1.
- An elution step was performed, again doing the displacement. This was performed from top to bottom. Subsequently, the redispersion was performed again from bottom to top at 240 l/h.
- Finally, a washing step was done while the elution liquid containing the protein was collected. To make sure displacement was efficient, the conductivity was measured and displacement was continued until the conductivity of the effluent undercut 3 mS/cm.

SolPro and MIEX DOC were easily transferred from the MEC by reversing the flow and flushing with water. Draining was not possible because of the design of the centrifuge. Displacement by an elution buffer was performed from bottom to top, while the elution buffer itself was displaced in opposite direction to take profit of the density difference. Conductivity measurement showed efficient displacement. The complete process is shown in Fig. 12.5.

The particle loss during separation was below the detection limit during the separation for all three particle kinds tested. The only significant particle loss appeared hence at cleaning after the process. Merck TMAP particles showed to form a stable cake in the center of the centrifuge which seemed to be lost for the process. This reduced the amount of separated protein significantly, the effect

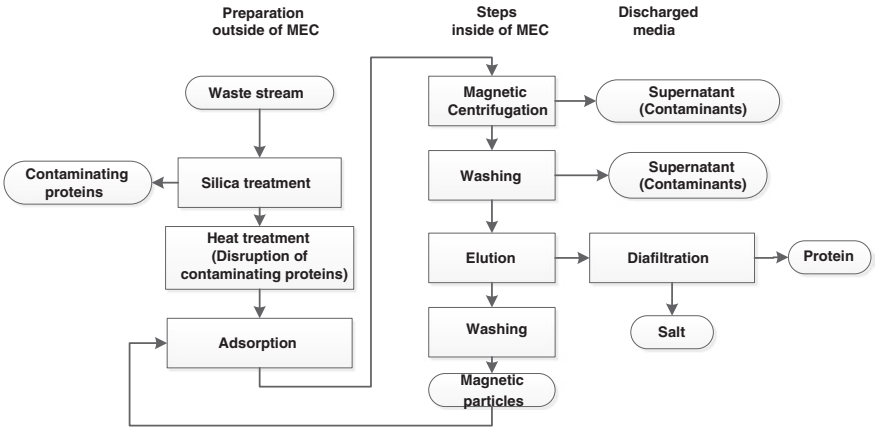

Fig. 12.5 The flow scheme of the BBI separation process shows the preparation steps outside of the MEC, the steps inside of the batch-wise MEC and lists the discharged media

Table 12.3 Separation efficiency of MEC with two batches of TMAP determined from particles in the eluates; trials on 300 g particles; Cycle 4 and 5 based on draining

Cycle number	BBI Mass (mg)	Separation efficiency (%)
1	18.5	99.994
2	64.8	99.98
3	63.5	99.98
4	11.7	99.996
5	33.9	99.98
6	63.2	99.97
7	84.6	99.97
8	96.5	99.97
9	80.1	99.97

was not observed in other particle systems. The BBI production trials comprised the use of Magprep TMAP in five cycles, Orica MIEX DOC in five cycles and SolPro PAAm in two cycles. Before cold storage, the eluates were preserved with 0.050 % Proclin. The separation efficiency of MagPrep TMAP is shown in Table 12.3.

12.4.3 Postprocessing: Purification and Drying

The eluate is dilute and contains a significant amount of salt. It was collected and filtered through a 0.2 µm filter. An iron content of 1.3 ppm was determined (4.7 ppm before filtration). As this is the same level as the original soy whey, magnetic particles seem not left in the eluate. Subsequently, the eluate was concentrated on a M20 membrane filtration rig using UFX10-pHt crossflow flat

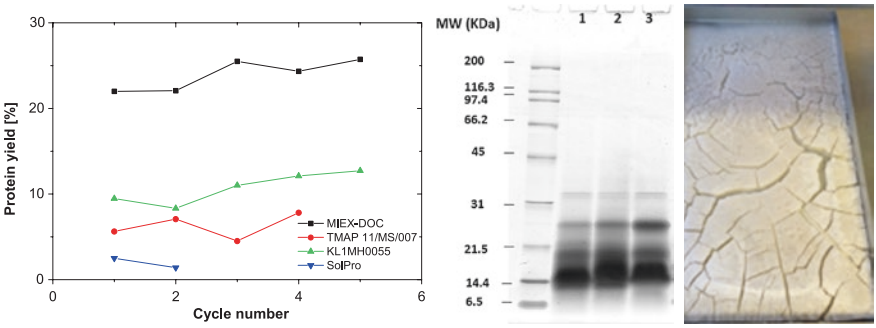

Fig. 12.6 *Left* the yield from all the binding-elution cycles run in the *food pilot line*, intended to identify if the particles show decreasing binding capacity; *middle* SDS gel of the three products from the *food pilot line*. The numbers refer to *1* TMAP, *2* MIEX DOC, *3* SolPro; *right* the product after freeze drying

Table 12.4 The stepwise protein and BBI yield is given, as well as the mass of protein in each step and the amount of BBI (Chymotrypsin inhibitor units (U))

	TMAP		MIEX DOC		SolPro	
	Protein (g)	BBI (u)	Protein (g)	BBI (u)	Protein (g)	BBI (u)
Soy whey content	243	63585	110	35325	49	7065
Eluate content	21 (8.7 %)	4745 (7.5 %)	26 (24 %)	8,870 (25 %)	1.0 (2.0 %)	132 (1.9 %)
Concentrate content	12 (57 %)	3435 (72 %)	13 (51 %)	6,930 (78 %)	0.6 (63 %)	25 (19 %)
Diawashed concentrate (g) or (U)	10 (86 %)	2,521 (73 %)	10 (77 %)	4,805 (69 %)	0.5 (83 %)	27 (108 %)
Dried powder content	11 (4.3 %)	2,280 (3.6 %)	11 (10 %)	4,325 (12 %)	0.4 (0.9 %)	20 (0.3 %)

sheet membranes from Alfa Laval at 50 °C. The eluate was concentrated to 3.5 L and diafiltered to less than 0.5 mS/cm. The seven times diawashing resulted hence in more than 1,000 times dilution. The diawashed concentrate was stored. Figure 12.6 C shows the result. The stepwise and total yields for all cycles of each of the three particle types are given in Table 12.4. There were protein losses in the order of 50 % in ultrafiltration and diawashing, reducing the overall yield.

To estimate the success of the purifications, the soy whey and the eluted fractions were analyzed by a chymotrypsin inhibition assay to determine the amount of BBI in units (CI/l). The amount of total protein was analyzed by a BCA assay and the specific activity (CI/g) and a fold purification of BBI could thus be calculated. Purification of BBI using magnetic adsorbents (Orica MIEX DOC and MagPrep TMAP) in pretreated soy whey was conducted in pilot scale (15 L soy whey, 300 g particles, 5 cycles) at using a MEC for separation (built by ANDRITZ

Table 12.5 Amount of product, protein content, activity and specific activity of each product

Particle	Amount of product (g)	BCA protein (%)	CI activity (U/g)	Specific activity (U/g protein)	Sugar (%)	Ash (%)	Kjeldahl protein (%)	ELIZA BBI (mg/g)
TMAP	22.2	47.3	108.5	229	14.1	14.5	61.5	72
MIEX DOC	19.3	56.6	224.1	396	8.7	12	66.8	159
SolPro	1.11	37.4	18.2	49	5.8	n/a	n/a	14

Contents of products, sugar determined by phenol-sulfuric acid method, ash by heating to 600 °C, Kjeldahl protein from nitrogen(*6,25) and BBI contents measured by FZMB

Table 12.6 Sugars found in the three dried products

Sugar	Stachyose	Glucose	Xylose	Sucrose	Galacturonic	Total (ppm)
TMAP	221.4	22.4	11.3	120.2	85.9	461.1
MIEX DOC	72.6	0.0	7.0	110.4	132.5	322.5
SolPro	60.5	0.0	3.5	74.3	83.6	221.8

KMPT GmbH). The BBI purifications in pilot trials reached a fold purification of 1.05 and 0.79 and a BBI yield of 25.1 and 8.5 % for Orica MIEX DOC and MagPrep TMAP, respectively.

In the lab MagPrep TMAP and MIEX DOC particles have shown to maintain their binding capacity through 50 cycles. Figure 12.6 left shows the yield per cycle of several particles in the process. As the yield is not reduced, the ligands stay active. Figure 12.6 middle shows protein contaminants in the powdered products. Figure 12.6 right shows the dried BBI product.

The amount of product produced varies, which is shown in Table 12.5.

Table 12.6 gives an overview over content of sugar. The sugars and ash numbers are high considering that excessive diafiltration was done. The samples have been filtered, and are precipitate-free, even though the color of the liquid sample is dark brown. The ash may be a result of chemical elements in the proteins not evaporating after burning, e.g., sulfur. The table shows the 200–500 ppm mono-di- and trimeric sugars, with the total content of 60,000–140,000 ppm carbohydrates. MS analysis has identified a small amount of glycoproteins and lectins.

There is a dense band on the gel for each product in Fig. 12.7, above the 180 kDa marker. This hints to soluble, negatively charged polysaccharides. These are known to be extracted from the soy bean during processing. The pectin-like molecules are negatively charged and might well be the contaminant competing contaminant reducing the amount of BBI separated in the process. Polysaccharides are also concentrated with the protein on UF, explaining the high sugar concentration.

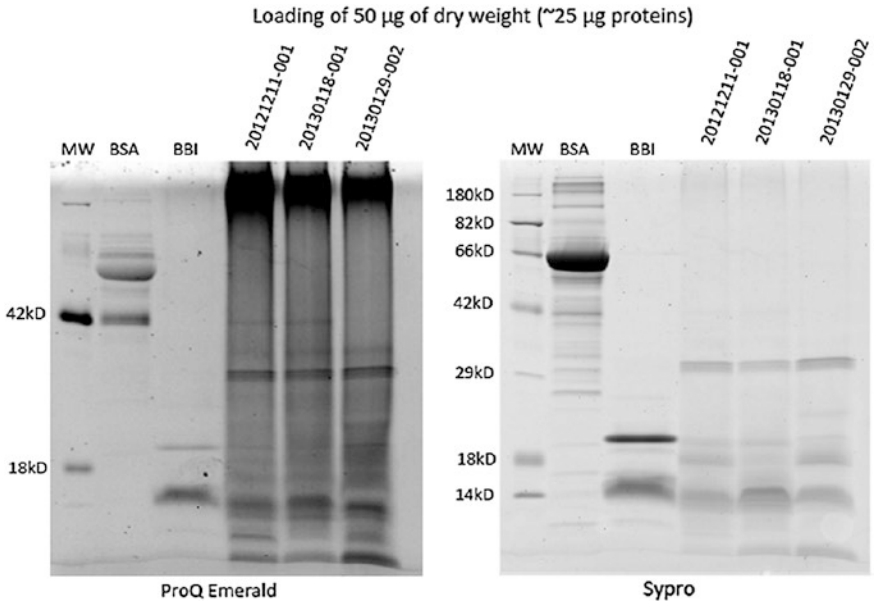

Fig. 12.7 *Left* gel stained targeting carbohydrates on glycoproteins; *right* gel stained targeting all proteins

12.5 Economic Evaluation

The main target of this chapter is the economic perspective of an industrial process. Therefore based on the separation experiments, a model calculation is performed to determine the economics.

12.5.1 Economic Process Modeling

The economic evaluation of the food pilot line was set up in the software SuperPro Designer®. The model was a direct implementation of the data of the batch-wise pilot trials of the food pilot line-based Orica MIEX DOC test runs. The materials, labor, and equipment costs, as well as utilities used in the trials were included to show the actual costs of the pilot scale process. These models serve as initial mass balance for the production scale models of the two processes. Three major assumptions are taken: .

1. All operations are continuous, running 300 days/year, 24 h a day, with a 1 week CIP interval.
2. The particles path in the process is changed into a continuous cycle of two continuous MEC units, one for separation after binding, the other after elution. These two units are extrapolated in cost, capabilities, and consumption from the pilot MEC200 from AKMPT.

3. Spray drying replaces freeze drying, because this batch-wise, laborious process is rarely scaled to production size.

Besides these changes, two additional assumptions are taken.

1. A membrane with insignificant loss of protein is identified and used for the purification.
2. Some form of separation aid is added, improving the separation during pretreatment and allowing for easier filtration.

The prices and utility requirements of all process units, including depreciation and prices of utilities and materials were identified and an industrially feasible process scheme created to enable a realistic evaluation of the process in production scale. A cost factor of 4.0 was used to find the total investment cost based on the equipment purchase price. The purchase prices of the equipment are scaled using the six-tenths rule, i.e., the approximate equipment cost C_B is estimated from the equipment cost at lower scale C_A, the size at lower scale S_A and the size at final scale S_B.

$$C_B = C_A \left(\frac{S_b}{S_A} \right)^{0.6} \tag{12.1}$$

The scale of the model is defined by the feed rate of 24 m^3/h of soy whey. A sensitivity analysis of the changing of key parameters in the production model is given below.

12.5.2 Process Economy

An economical model was created based on the test production runs of Orica particles, because this system achieved in comparison high separation rates at a low particle price. Technically an upscale of the other particle systems would result as well in lower market prices. Calculations were performed with the assumption of avoiding further losses during washing and drying, resulting in 25 % separation actually achieved in the pilot plant. Current losses result in doubling the production cost given below. The BBI yield of Orica particles of 25 % resulted in a production rate of 73.5 ton of spray dried product per year. The product contained a BBI activity of 270 U/g. The calculation resulted in costs of 344 €/kg in a large scale production line. The same scenario for TMAP yields 8.5 % BBI, with a production of 30.7 ton per year at an activity of 220 U/g. The costs of this product are higher, reaching 5,910 €/kg.

In the sensitivity analysis, a separation of 25 % of the protein was assumed (which comes at no cost in a waste stream) at 24 m^3/h with particles at 2 mass % concentration of 254 €/kg price with particles used for 100 cycles (i.e., low loss and no deterioration at these 100 cycles).

Figure 12.8 left shows the cost distribution of the Orica production process. The largest expense is the replacement of particles, followed by investment depreciation (over a 10 year period) and facility costs. The pretreatment of the soy whey

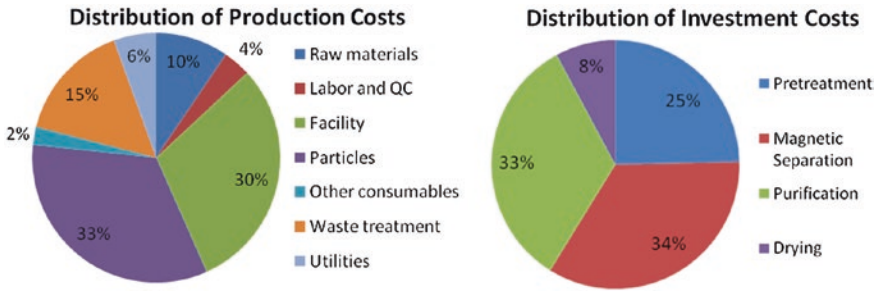

Fig. 12.8 *Left* distribution of production costs for a plant based on Orica particles(25 million €/yr); *right* distribution of investment costs for a plant based on Orica particles (25 million €)

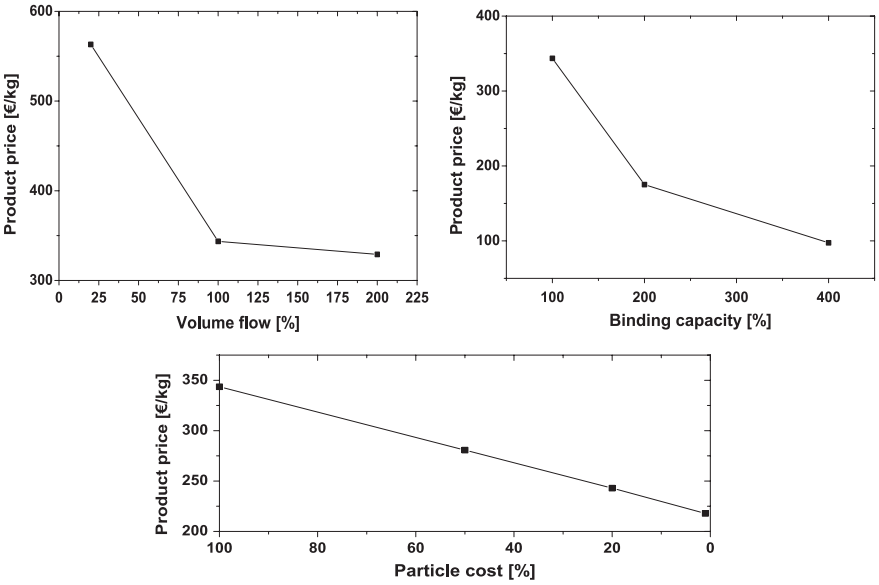

Fig. 12.9 *Top left* the effect of process capacity on product price, relative to the reference process model. *Top right* the effect of binding capacity on product price, relative to the binding capacity identified in the pilot trials. The models assume that higher binding capacity results in higher process yield, as the process parameters remains constant. *Bottom* The effect of particle cost on the product price, relative to the price indicated by Orica for MIEX DOC particles

for the process accounted for the third largest costs proportion in the calculation, as both the purchase and disposal of the solid waste showed to be significant in a process. Utilities, consumables, and chemicals represent only minor expenses compared to the factors aforementioned. Figure 12.8 right shows that the equipment costs are dominated by the first steps of the process handling a large volume. In the pretreatment, the decanter centrifuge and in the magnetic separation the MEC and UF showed to be important, while spray drying is cheap due to low volume.

Figure 12.9 bottom shows the influence of the particle cost and the consequence of a theoretic reduction. Figure 12.9 top right shows a theoretic increase of

the process yield by a higher binding capacity, which would reduce the cost even more with a theoretic 90 €/kg at a protein yield of 100 %. A theoretic evaluation of a further increase of the process scale in Fig. 12.9 top left does not improve the process significantly due to its already very high scale in the calculation. Omitting the Silica pretreatment step has been shown in the lab to have minor impact on the yield but reduced the production costs to 276 €/kg.

In this study, even with further developments and optimizations the limit of a production cost of BBI proteins can be seen at 50 €/kg.

12.5.3 Theoretic Calculation on Selective Ligands

A theoretic study was as well run on the production model by highly selective MagPrep Antibody particles at 49 €/g functionalized particle and 100 cycle times. The result was a price of 180,000 €/kg product, while Orica product resulted in 340 €/kg. A consequence is that the nonparticle related cost sum to only 0.33 %, which is neglectable. Orica reach about 270 U/g(~150–200 mg/g), while antibody functionalized particles might in theory achieve above 1,000 U/g including a washing step. In a pure product, the price would be 270,000 €/kg. A high yield and a selective process are in the calculation essential to product purity. The price of the magnetic fishing technology using antibodies is estimated exclusively by the antibody particle cost C in €/g, the binding capacity B in (g/g) and lifetime L in cycles which includes as well particle losses in the separation. The particle cost constitutes more than 90 %, for specific particles more than 99 %, of the total costs in industrial scale systems. To give an example, the cost of 1 kg BBI produced by particles at 49,000 €/kg at a binding capacity of 2 mg/g and a lifetime of 100 cycles results in 245 €/g.

$$C = \frac{P}{B * L} \tag{2}$$

In conclusion, the process yield and fold purification is highly dependent on the binding and elution technology in the process. The binding capacity, and thereby process yield, together with the lifetime and cost of the resin/magnetic particles, are important cost drivers. Further optimization is possible both resin- and process-wise, but magnetic adsorbent-based purification using a MEC is competitive to other adsorption technologies and can be expected to show additional benefit by a reduction of pretreatment of the feedstocks. In the model ultrafiltration seems to be the most cost-efficient process, while not being highly selective. In comparison to conventional adsorption technologies, magnetic fishing seems to be a commercially viable option.

12.6 Technology Benchmarking

A theoretic benchmark against established competing technologies for the production of specific proteins from coproduct streams allows estimating the commercial perspectives of the process. Relevant competing technologies are

packed bed adsorption (PBA), expanded bed adsorption (EBA), and ultrafiltration (UF). The benchmarking is based on lab experiments at the assumption that separation achieved in lab experiments is scalable (EBA, PBA, UF, HGMF).

PBA bases on adsorbents immobilized in a packed bed, which is passed by the target liquid. EBA is a variant of PBA, in this case the packed bed is passed from bottom to top at flow velocity sufficient to equalize gravity and resistance force of the packing to create a fluidized bed. This results in a lower necessary pretreatment, as larger contamination passes unhindered. PBA has several of the advantages of HGMF, but is not possible in a continuous way. UF is a common semipermeable membrane-based separation selective for molecular weight instead of surface properties. This is rather different compared to HGMF, PBA, and EBA, which are adsorption-based separation methods.

The benchmark of performance and costs of the technology is based on ion-exchange resins. In this comparison, two magnetic adsorbent carrier systems (Orica DOC and MagPrep TMAP) are compared to three established separation technologies (EBA, PBA, and UF) for the purification of the target protein BBI from soy whey. The modeling of the five benchmark processes included the feedstock pretreatment and the downstream processing in the software SuperPro Designer®. The mass was balanced and the equipment sized according to the process scale. The economic consequences of various manufacturing scenarios were evaluated, and prices for materials (equipment, utilities, and chemicals), energy, and labor were incorporated into the process models to calculate costs based on pilot scale trials.

12.6.1 Experimental Test Runs on Competing Technologies

The purification of BBI in soy whey was developed in both EBA and PBA with approximately 300 mL of pretreated soy whey. The activity of the pretreated whey and the eluted samples containing protein were analyzed with the chymotrypsin inhibition assay to quantify BBI and BCA assay. The purification of PBA and EBA resulted in a fold purification of 1.2 and 0.97 and a BBI yield of 18 and 11 %, respectively (Table 12.7). The UF membrane used in the benchmarking study had a cut-off of 3 kDa and can be expected to retain the BBI in the retentate as BBI has a molecular weight of 8 kDa. UF of pretreated soy whey resulted in a fold purification of 1.61 (Table 12.7) which is higher compared to both EBA and PBA. The yield of BBI was 81 %. The fold concentration of BBI in the retentate from UF is 5.28 which shows that most of the proteins present in the soy whey were retained in the retentate. EBA was chosen as a promising technology to benchmark against magnetic adsorbents as both EBA and magnetic adsorbents can handle purification in unclarified feed stocks which neither packed bed adsorption (PBA), chromatography nor ultrafiltration

Table 12.7 PBA, EBA, UF and HGMF purification of BBI from pretreated whey

PBA	Vol. mL	CI/L	CI/g	% BBI yield	Fold conc.	Fold purification
SW load	304	324	181	100	1.00	1.00
Elution	20	894	217	18	2.76	1.20
EBA						
SW load	302	324	141	100	1.00	1.00
Elution	36	297	135	11	0.92	0.97
SW load	110	348	229	100	1.00	1.00
Elution	27	943	597	66	2.71	2.60
UF						
SW load	100	448	201	100	1.00	1.00
Retentate	15.4	2367	324	81	5.28	1.61
HGMF (Orica)						
SW load	15,000	471	320	100	1.00	1.00
Retentate	16,042	111	337	25.1	0.24	1.05

(UF) do. A fold purification of 2.6 at 110 mL soy whey load was obtained. It was possible to use food compatible buffers in the process without affecting the results. While 300 mL soy whey in an EBA column did not deliver good results, an optimal amount of pretreated soy whey (110 mL) was identified. This corresponds to 7 times the column volume to reach a high yield and amount of purified BBI.

12.6.2 Benchmarking Results

The models comprise all steps of the process, from pretreatment of the soy whey to drying of the purified and concentrated product. The mass balances were scaled linearly. In addition the pretreatment, purification, and drying processes were adjusted to yield products of similar purity. This allows for the cost differences in the investigated processes.

EBA was in our calculation efficient in yield and thereby utilization of the equipment and resources, giving a good cost optimization potential. The influence might as well be down to a better optimization. The magnetic fishing process based on Orica particles showed to be highly cost efficient. The costs of BBI production shown in Fig. 12.10 can be compared to the price of the currently commercially available BBI from the Sigma Aldrich catalogue (3000 €/g). The project purity goal of more than 1,000 U/g was not reached in the experiments. While the current cost estimate appears low at less than 20 €/g, the result is rather theoretic as long as no particles for a purity increase are found.

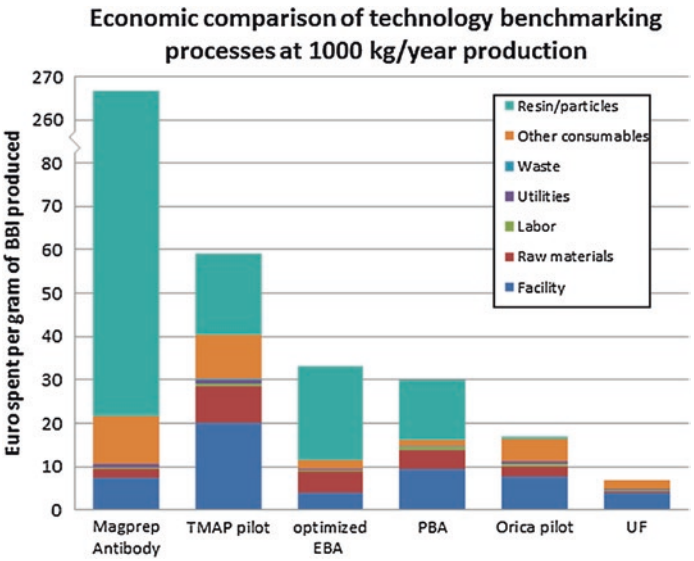

Fig. 12.10 Comparison of the costs of 1 g BBI distributed on major cost items for the five key models in the technology benchmarking

12.6.3 Carbon Footprint

The life cycle assessment investigates the environmental implications of BBI separation. The Life Cycle Assessment (LCA) is done according to ISO 14040 and ISO 14044. The assessment compares BBI separation by EBA with BBI separation by the novel magnetic separation process. Two magnetic separation scenarios were investigated. The first magnetic separation scenario is based on lower yields than currently obtained in pilot scale experiments, while the second scenario assumes similar BBI separation yields compared to the expanded bed adsorption reference case. Due to the high specific energy and material consumption of the separation processes, the separation of BBI by either EBA or magnetic separation has a significant environmental burden. Assuming comparable BBI separation yields, the magnetic separation process seems to be superior to the expanded bed adsorption process with respect to the environmental categories studied in the assessment (MEC+TMAP I in Fig. 12.11). At low yields, currently observed in the experiments, expanded bed adsorption would be the more sustainable BBI separation process choice with respect to the environmental impact categories assessed in this study (MEC+TMAP II in Fig. 12.11).

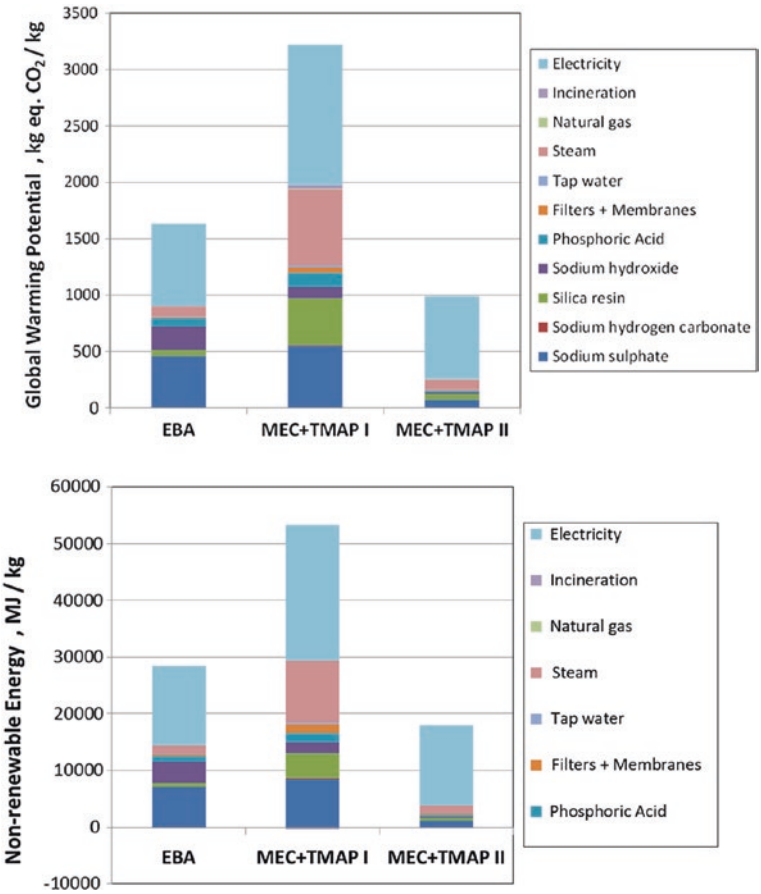

Fig. 12.11 *Top* benchmark comparison on the climate change; *bottom* benchmark Comparison on nonrenewable energy consumption

12.7 Safety, Health and Environment and Legal Considerations

On October 18th 2011, the European Commission adopted the Recommendation on the definition of a nanomaterial. According to this recommendation a "Nanomaterial" means:

> A natural, incidental, or manufactured material containing particles, in an unbound state or as an aggregate or as an agglomerate and where, for 50 % or more of the particles in the number size distribution, one or more external dimensions is in the size range 1–100 nm.

Nanomaterials are not intrinsically hazardous per se but there may be a need to take into account specific considerations in their risk assessment. The chosen Orica particle system was not considered as nanomaterial.

12.7.1 Nanomaterials in REACH and CLP

REACH is the over-arching legislation applicable to the manufacture, placing on the market and use of substances on their own, in preparations or in articles. Nanomaterials are covered by the definition of a "substance" in REACH, even though there is no explicit reference to nanomaterials.

In the European Commission the definition of a nanoparticle, the determination of the percentage of particles in the number size distribution is required. Currently, the exact determination of the size distribution is difficult because of methodological limitations. **B**runauer-**E**mmett-**T**eller (BET) allows the determination of the specific surface, and a deduced average particle size, but not a number size distribution. **D**ynamic **L**ight **S**cattering (DLS) results in a size distribution, but cannot distinguish between individual particles and aggregates. The most precise method would be the evaluation of electron micrographs (SEM, TEM) by directly measuring the diameter of a large number of particles. Manual screening of hundreds or thousands of particles would be too time-consuming and expensive.

12.7.2 Potential Risks/Negatives of the Nanomaterial

Dermal irritation and dermal sensitization studies were conducted with MagPrep TMAP test materials. In those studies, the test substance was a skin irritant (OECD 404) or a dermal sensitizer (OECD 429). Acute oral toxicity studies were also conducted with a group of female rats (OECD 423). Under the conditions of the study, single oral applications (2 g/kg body weight) of the MagPrep TMAP test substance produced no mortality and were associated with minor signs of toxicity, which were fully reversible within 1 day postexposure. Because all of the exposed female rats survived the single dose through a 14 day postexposure period, the median lethal dose (LD_{50} cut-off rat) of particles is potentially at 5 g/kg. The magnetic particles, which go to the municipal wastewater treatment plant are expected to partition to washed grit/sand.

In conformity with the criteria given in Annex VI to Commission Directive 2001/59/EC, the test item has no obligatory labeling requirement for toxicity. According to Annex I of Regulation (EC) 1272/2008, the test item MagPrep TMAP has no obligatory labeling requirement for toxicity and is not classified. According to Globally Harmonized Classification System (GHS) the test item has obligatory labeling requirement for toxicity and is classified into Category 5: "LD50 >2,000 mg/kg <5,000 mg/kg. WARNING. No symbol. May be harmful if swallowed."

12.8 Conclusion

A pilot line was set up, processing magnetic particles at losses of less than 1 ‰ particles. The Achilles heel of the process is the lack of selective ligands, which resulted in competing adsorption of contamination and in a less efficient process. The specific activity did not excel 400 U/g protein, or 159 mg/g product. Additional development is specifically necessary to achieve commercially interesting concentrations of BBI.

Combining actual pilot scale measurements with detailed modeling showed that a product holding BBI equal to 270 U/g could probably be produced at a cost in the range of 344 €/kg. The cost is sensitive to the magnetic fishing step in particular. Different particle kinds are available, with price being a major criterion. The separation device as the core of a pilot line showed to be necessary in a continuous version for large scale use. A realization of the simulated process scale is tempting for performance. It depends on the availability of selective ligands at large scale.

The risk assessment characterizes the human health risk and environmental risk as low when handling the investigated materials.

In conclusion, there are several apparatuses available or in development which allow HGMF at a scale from µl-batches to continuous production at a scale of 1 m^3/h. Systems for magnetic particles are in development and on the market, with a wide spread in price and properties. Particle systems as cheap as 250 €/kg were found and protein production costs of below 50 €/kg were reported. Both particle and separation device seem to be in an evolved state for use. The comparison of different separation technologies shows a large commercial potential for magnetic separation in comparison to standard separation technologies.

Critical for the process is the specific system and the ligands. In the system, the contaminations and the target product influences the choice of the ligand. The identification of a ligand, which separates the target selectively, can be eluded and recycled and is available at reasonable costs is the main challenge. It needs to be fitted to the system, so there is not general approach. Despite major effort in the current project, a selective ligand for the particle system could not be brought to large scale. Less selective ligands showed competing adsorption, which reduced the efficiency of the whole process.