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The influence of cell adsorbent interactions on protein adsorption in expanded beds

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Abstract

Expanded bed adsorption (EBA) is a primary recovery operation allowing the adsorption of proteins directly from unclarified feedstock, e.g. culture suspensions, homogenates or crude extracts. Thus solid–liquid separation is combined with adsorptive purification in a single step. The concept of integration requires that the solid components of the feed solution are regarded as a part of the process, which influences stability, reproducibility, and overall performance. This aspect is investigated here at the example of the influence of presence and concentration of intact yeast cells (*S. cerevisiae*) on the adsorption of model proteins (hen egg white lysozyme and bovine serum albumin) to various stationary phases (cation and anion-exchange, hydrophobic interaction, immobilised metal affinity). The interaction of the cells with the adsorbents is determined qualitatively and quantitatively by a pulse response method as well as by a finite bath technique under different operating conditions. The consequence of these interactions for the stability of expanded beds in suspensions of varying cell concentration is measured by residence time distributions (RTDs) after tracer pulse injection (NaBr, LiCl). Analysis of the measured RTD by the PDE model allows the calculation of the fraction of perfectly fluidised bed (φ), a parameter which may be regarded as a critical quantity for the estimation of the quality of fluidisation of adsorbents in cell containing suspensions. The correlation between bed stability and performance is made by analysing the breakthrough of model proteins during adsorption from unclarified yeast culture broth. A clear relationship is found between the degree of cell/adsorbent interaction, bed stability in terms of the φ parameter, and the sorption efficiency. Only beds characterised by a φ value larger than 0.8 in the presence of cells will show a conserved performance compared to adsorption from cell free solutions. A drop in φ, which is due to interactions of the fluidised adsorbent particles with cells from the feed, will directly result in a reduced breakthrough efficiency. The data presented highlight the importance of including the potential interaction of solid feedstock components and the expanded adsorbents into the design of EBA processes, as the interrelation found here is a key factor for the overall performance of EBA as a truly integrated operation.

Keywords: Adsorption; Expanded bed adsorption; Residence time distribution; Cell adhesion; Proteins

1. Introduction

Expanded bed adsorption has been introduced in order to combine solid–liquid separation and adsorption in a single step [1,2]. A suitable stationary phase is fluidised by applying the mobile phase in an upward direction thus yielding a bed of increased interparticle distance. The enlarged void fraction of the fluidised bed allows the application of particle containing feedstock without the risk of blocking the

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bed. Thus adsorptive protein purification becomes possible starting from biomass containing suspensions. When adsorbent particles with a wide size distribution ($2 \cdot d_{pmin} < d_{pmax}$) are employed, a classified fluidised bed with reduced mixing is formed and adsorptive processes in these systems are termed expanded bed adsorption (EBA). It has been shown successfully, that adsorption of proteins in stable expanded beds is governed by similar process parameters as packed bed protein adsorption. Provided particulate fluidisation is attained and axial mixing is reduced due to classification of fluidised adsorbent particles, external and internal transport processes are dominating the sorption performance thus making EBA a residence time controlled process [3–5]. As long as these conditions are met, sorption performance in the stable expanded bed can be anticipated to be similar to packed bed operation.

As the concept of protein adsorption in expanded beds was introduced in order to allow protein purification starting from unclarified feedstock, one major question in EBA is, whether the stable expanded bed condition is met, when particle containing solutions as culture suspensions or homogenates are employed for fluidisation of adsorbent particles. Despite the importance of this point there is only very little information available on the influence of the complex biological feedstock on process performance. In the early work of Chase and co-workers the influence of cells on equilibrium and kinetics of protein adsorption to fluidised porous particles was studied [6,7]. Additionally some qualitative information was given by Barnfield-Frej et al. on the influence of *E. coli* homogenate on EBA performance [8] and by Erickson et al. on EBA stability in the presence of mammalian cells [9].

In order to improve this situation three different pieces of information are needed:

(a) It has to be investigated whether, and to which extent, solid components from the feedstock (cells or cell debris) bind to the adsorbent under process conditions.

(b) Data have to be collected on whether this interaction leads to a deteriorated bed stability during fluidisation of the respective stationary phase in the real feed suspension.

(c) A quantitative correlation between bed stability under process conditions and adsorption performance has to be established.

Based on this information the process conditions can be optimised with regard to minimised interference of solid feed components with process performance.

Recently we have presented two techniques, which allow the characterisation of EBA with regard to bed stability under real process conditions. A pulse response method has been developed in order to characterise the interaction of fluidised adsorbents with suspended cells or homogenate particles [10]. With this method the transmission of a cell pulse through a stable expanded bed is measured and a deviation from 100% transmission is translated into an interaction between the biomass in the pulse and the fluidised adsorbent. Using this technique a variety of cell/adsorbent combinations was characterised with regard to potential adsorption of cells to the stationary phase under process conditions. Furthermore it was shown that the technique may be used for optimisation of process conditions regarding minimised cell/adsorbent interaction. The influence of these interactions on bed stability was investigated based on residence time distributions (RTDs) [11]. The response to pulses of fluorescent tracer molecules was measured when expanded beds were fluidised in complex biotechnological feedstock as culture suspensions and homogenates. The response signals were analysed with an advanced RTD model. According to the so called PDE model [12] the adsorbent bed was divided into two zones: (a) the dynamic (stable) zone where a perfectly classified and stable expanded bed exists and (b) the stagnant zone where aggregative fluidisation due to interactions between suspended solids in the feed and the adsorbent develops. Fig. 1 shows a schematic representation of the model, which is defined by three parameters, the fraction of stable expanded bed (φ), a mass transfer term between both zones (N) and a Peclet number (Pe) accounting for axial mixing in the stable zone. A φ value of 1 represents an ideal expanded bed with particulate fluidisation throughout the bed and virtually no channels or aggregates. High Pe numbers stand for reduced axial mixing in the bed, with values above 40 being uncritical for the performance of protein adsorption [13]. The parame-

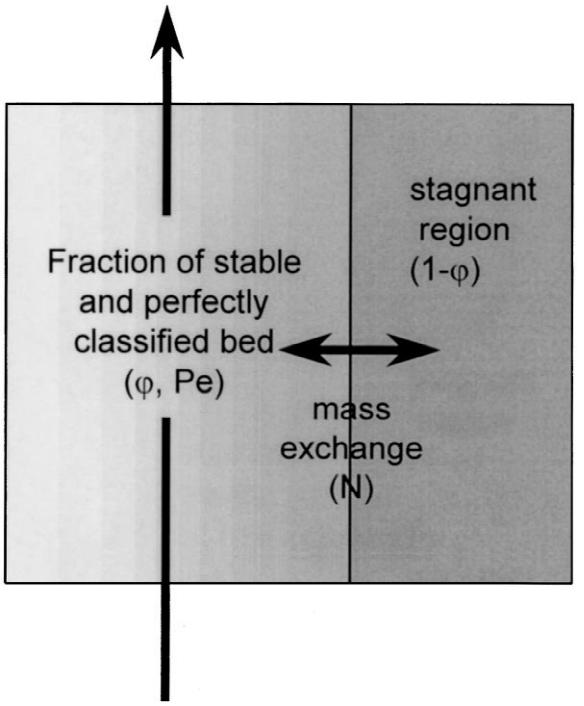


Fig. 1. Schematic representation of the PDE model.

ter φ may under these conditions be employed to estimate the usefulness of a certain expanded bed for protein adsorption: φ values below 0.8 describe a bed, where less than 80% of the bed fulfils the criterion of perfect classification and which must be supposed to have inferior sorption performance compared to stable expanded beds fluidised in particle free model solutions.

The purpose of this work is to investigate the influence of cell adsorbent interactions on the performance of EBA. Taking the adsorption of two model proteins, i.e. hen egg white lysozyme (HEWL) and bovine serum albumin (BSA), from suspensions containing intact yeast cells (*S. cerevisiae*) to several expanded adsorbents (Streamline DEAE, Streamline SP, Streamline Phenyl and Streamline Chelating, Amersham Pharmacia Biotech, Uppsala, Sweden) as an example, the aforementioned methods will be used in order to check the systems for potential interactions and bed stability. Sorption performance will be measured based on protein breakthrough starting from particle free model solutions as well as from yeast suspensions of varying solids concentration.

2. Materials and methods

2.1. Hardware, adsorbents, proteins, and process chemicals

Fraction V BSA and HEWL were purchased from ICN (Meckenheim, Germany), all other process chemicals were from E. Merck (Darmstadt, Germany). Streamline AC, Streamline DEAE, Streamline SP, Streamline Phenyl and Streamline Chelating are commercially available media for EBA and were obtained from Amersham Pharmacia Biotech (Freiburg, Germany). A laboratory-made column for EBA (1 m×0.02 m I.D.) was used. A small amount of glass ballotini (0.3 mm diameter; <5% total sedimented bed height) was added to improve flow distribution at the column inlet and a movable adapter was employed to adjust the position of the liquid outlet to the top of the expanded bed. The fluid was transported using a peristaltic pump (Abimed, Langenfeld, Germany) controlled by a gravimetric flow control unit (Sartorius, Göttingen, Germany). Proper column vertical alignment was assured in all the experiments. Protein concentration in particle free effluents was detected on-line by a flow-through UV monitor (Knauer, Berlin, Germany). Particle containing effluents were clarified by centrifugation (10 mm at 10 000 *g*) and analysed for protein content off-line by UV adsorption at 280 nm or by the Bradford procedure [14]. Experiments were run using phosphate based buffers; i.e., 20 m*M* potassium phosphate buffer pH 7.2 for ion-exchangers (Streamline DEAE, Streamline SP), the same buffer with ammonium sulphate (1.0 *M*) added for Streamline Phenyl, and 0.25 *M* sodium chloride containing buffer (adjusted to pH 7.6) for Streamline Chelating. The latter was loaded with copper ions (2+) by exposure, either in bath or column mode, to a 5% copper sulphate solution and subsequent wash with distilled water.

2.2. Analysis of cell/adsorbent interaction by the 'biomass impulse' method

The respective adsorbent ($L=0.12$ m) was fluidised at $8.3\cdot10^{-4}$ m s^{-1} in 20 m*M* potassium phosphate based buffers pH 7.2. Sodium chloride was added to the buffer in some experiments at varying final concentrations. 40 ml pulses of intact yeast cell suspension (0.1% wet mass) were injected to the system and analysed before and after the column by UV/VIS adsorption. Pulse transmission was measured by comparing the pulse area before and after passage through the column as described elsewhere [10].

2.3. Analysis of cell/adsorbent interaction by finite bath experiments

The degree of cell adsorption to the stationary phases was evaluated from finite bath experiments in a mixing vessel over a prolonged period of time. A suspension of intact yeast cells (0.1% wet mass) in potassium phosphate based buffer (20 m*M*, pH 7.2) was stirred gently (hanging stirrer, impeller diameter 0.03 m; 100 rpm; calculated Re_A 1440) in a 1.0 litre beaker. Sodium azide (0.01%) was added to the buffers to prevent microbial growth during the course of the experiments. The suspended biomass was re-circulated with a peristaltic pump (Verder, Düsseldorf, Germany) at a flow-rate of 1.5 l h^{-1} through the flow cell of a UV/Vis detector (Knauer, Berlin, Germany). Once equilibrated in buffer, the adsorbent (25 ml) was added to the system and the decrease in adsorption at 600 nm was followed during 12 h using a personal computer with data acquisition software. Interference from adsorbent particles in the bulk with the absorbance measurement was avoided by introducing a fine net to the liquid aspiration probe preventing adsorbent beads from entering the detector. Under the experimental conditions described no build up of biomass deposits was noticed in any part of the system. Experiments were carried out at 25°C.

2.4. RTD measurements in the presence of cells

RTD measurements were performed using a pulse-response method. Experiments were carried out with 0.16 m sedimented bed and at a fluid velocity of $5.3\cdot10^{-4}$ m s^{-1}. After equilibrating the bed with the appropriate mobile phase (a suspension of varying concentration of *S. cerevisiae* in 20 m*M* phosphate based buffers), a pulse (500 μl) of 1.0% solution of NaBr (or LiCl) in buffer was injected at the inlet of the column with a sample motor valve (Besta, Wilhelmsfeld, Germany). The ionic environment in these buffers was adjusted using 50 m*M* sodium nitrate (or 50 m*M* ammonium sulphate) in order to obtain a constant signal background. Combination Glass Body Electrodes (Cole-Palmer, Niles, IL, USA)-installed in an appropriate flow cell-and a pH/mV meter (LKB, Bromma, Sweden) were used for the direct measurement of Bromide and Lithium ions (tracers) in biomass containing feedstock. A small concentration of the target ion (10^{-5} *M*) was also included in the buffer composition to assure linear (in logarithmic scale) and fast enough (response time ≤10 s) system response. The response signals were detected by the on-line ion-selective-electrodes (ISEs), translated into a RTD function, and analysed by the PDE model. Model parameters were obtained by fitting the experimental curves to the theoretical model in the Laplace domain using the expression originally developed by Villermaux and van Swaiij [12]:

$$\bar{C}(s) = L[E(\theta)] = \frac{\sqrt{Pe}\cdot\exp\left(\frac{Pe}{2}\right)\cdot\exp\left[-\sqrt{Pe\cdot\varphi}\cdot\sqrt{s+\frac{N}{\varphi}+\frac{Pe\cdot\varphi}{4}-\frac{\frac{N^2(1-\varphi)}{\varphi}}{s+\frac{N}{(1-\varphi)}}}\right]}{\frac{\sqrt{Pe}}{2}+\sqrt{\varphi}\cdot\sqrt{s+\frac{N}{\varphi}+\frac{Pe\cdot\varphi}{4}-\frac{\frac{N^2(1-\varphi)}{\varphi}}{s+\frac{N}{(1-\varphi)}}}} \quad (1)$$

Back transformation to the time domain was performed using a commercial software package (Scientist, Micromath, Salt Lake City, UT, USA). Details on the procedure and its application to RTD analysis in expanded beds has been published elsewhere [11].

2.5. Isotherm for adsorption of S. cerevisiae to stationary phases

A fluid volume (V_l) of 10 ml of yeast cell

suspension (0.06% to 15% wet mass in 20 m*M* phosphate based buffers) was incubated for 24 h with 1 ml adsorbent volume (V_s) of the stationary phase under investigation. The cell concentration in the liquid phase was determined by UV absorption at 600 nm or by cell counting at the beginning and the end of the incubation phase. The apparent capacity of the adsorbent for the yeast cells was determined according to:

$$Q = \frac{(C_{\text{cells0}} - C_{\text{cellsseq.}})V_l}{V_s} \quad (2)$$

Cell counting was performed with an industrial Coulter Counter Model D (Coulter Electronics, Luton, UK) equipped with a 100 μm orifice tube. Operational routines were executed according to the manufacturer recommendations, using 5 μm latex particles as size standard.

2.6. Breakthrough experiments

The respective adsorbent was filled into the column to 0.2 m sedimented bed length and was fluidised with at least 10 column volumes of 20 m*M* potassium phosphate based buffers pH 7.21 at $5.3 \cdot 10^{-4}$ m s^{-1}. After equilibration bed stability was investigated by measuring the RTD function of the bed as described above. A stable expanded bed was defined as being characterised by a φ value of >0.95 and *Pe* of more than 40 according to analysis by the PDE model. In case of adsorption of proteins from yeast cell suspension the bed was then equilibrated with a solution of the desired *S. cerevisiae* cell content. After equilibration the breakthrough experiment was started by application of a cell suspension of the same solids concentration containing 4.2 kg m^{-3} (or 1.5 kg m^{-3}) BSA in the case of Streamline DEAE (or Streamline Phenyl), and 3 kg m^{-3} (or 2.1 kg m^{-3}) HEWL in the case of Streamline SP (or Streamline Chelating). Protein concentration in the effluent was monitored and the experiment was stopped when $>95\%$ of the initial protein concentration was reached at the column outlet. The equilibrium capacity was determined by integration of the breakthrough curve as described by Yamamoto and Sano [15]. Sorption performance was expressed as the ratio of breakthrough capacity at 10% breakthrough to the equilibrium capacity.

3. Results and discussion

3.1. Cell–adsorbent interaction

Cell–adsorbent interaction was investigated by a variety of methods. The biomass pulse technique is able to evaluate cell adsorption to the fluidised solid phase under actual hydrodynamic (superficial velocity) conditions. The use of small amounts of cells in this test means that only the primary phase of a complex cell adsorption phenomenon can be observed (e.g., the interaction of single cells with the adsorbent at low coverage of the adsorbent particle surface).

Fig. 2a shows cell transmission values found for the different adsorbents tested in this work under standard experimental conditions. In order to investigate cell adsorption to the agarose backbone of the stationary phases used, a batch of adsorbent without any ligand was tested first. This material (Streamline AC) showed 100% cell pulse transmission demonstrating that the agarose based matrix is inert towards the complex biotechnological feedstock. For the system *S. cerevisiae*/Streamline SP at pH 7.2. almost 100% transmission of the yeast cells through a stable expanded bed was found, thus indicating that *S. cerevisiae* does not interact with the cation-exchanger under these conditions. This result had been expected on the basis of the colloid interaction theory, as at neutral pH yeast cells are negatively charged at their surface and are thus repelled from the also negatively charged stationary phase [16]. It has been demonstrated by particle electrophoresis and interaction with cationic compounds, that phosphate and carboxylate groups are the dominant functionalities determining the cell surface charge [17]. A very strong interaction was found with Streamline DEAE at the same pH and ionic strength as demonstrated by only 23% transmission of a yeast cell pulse through an expanded bed of the anion-exchanger. In this case electrostatic interactions between cells and stationary phase led to adsorption of *S. cerevisiae*. Cell pulse transmission could be improved by adding salt (NaCl) to the mobile phase

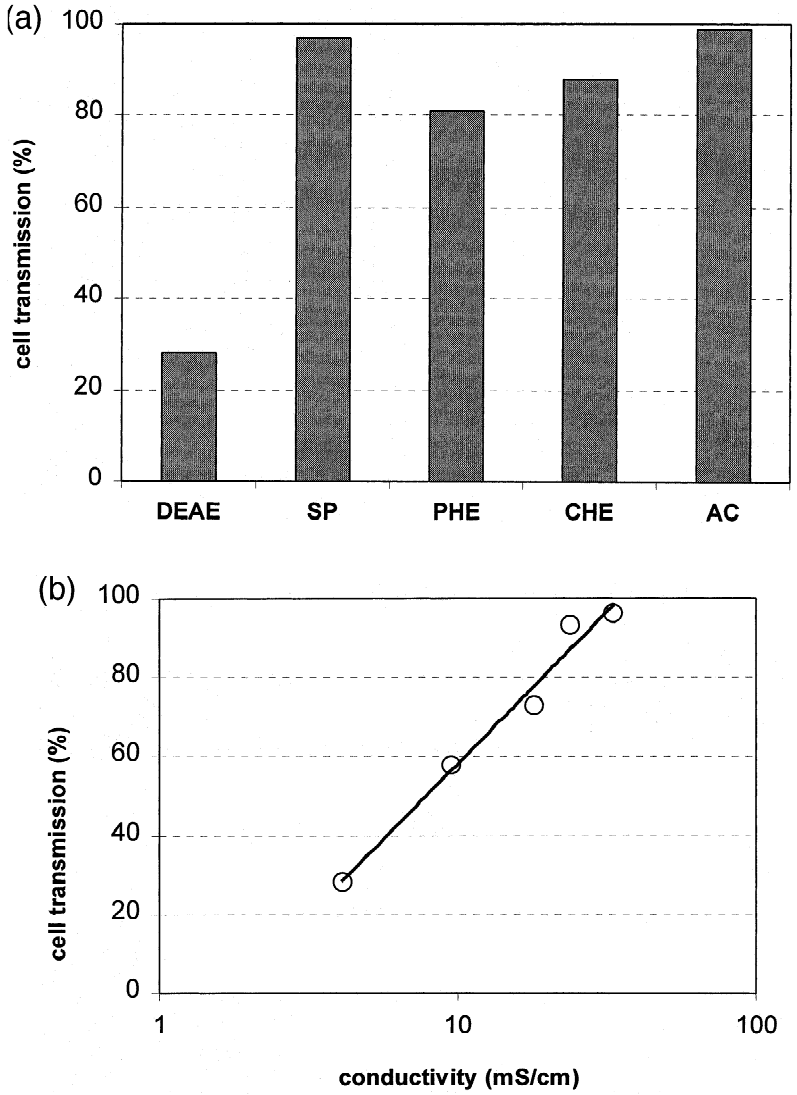


Fig. 2. (a) Cell pulse transmission values for intact yeast cells through expanded beds of Streamline adsorbents obtained with the 'biomass pulse' technique. Phosphate based buffers of standard composition for each chromatographic mode were employed. (b) Cell pulse transmission values for Streamline DEAE using yeast cells in phosphate buffer pH 7.2 at varying conductivity (adjusted by NaCl addition).

as shown in Fig. 2b. Apparently the adsorption of *S. cerevisiae* to the anion-exchanger is suppressed by the presence of small ions, an experimental finding which supports the previous assumption of ionic cell–adsorbent interactions. Good yeast cell transmission (>85%) was also found with Streamline Phenyl and Streamline Chelating (Cu^{2+}) adsorbents, showing that moderate to low interactions take place in both cases where ionic adsorption mechanisms are less likely. From these experiments it may be concluded that electrostatic forces are responsible for the initial uptake of biological particles and cells in expanded beds under real process conditions.

In order to further explore biomass adsorption behaviour finite bath (kinetic) experiments were also carried out. This complementary method involves a

prolonged contact time (up to 12 h) between the cell suspension and the adsorbent particles. Gentle mixing was provided ($Re_A \sim 1500$) below the full turbulent flow regime to avoid shear effects. Due to the prolonged residence time information is obtained on the 'secondary' or layering phase which is likely to occur at higher coverage based on the interaction between cells already immobilised on the adsorbent and other suspended biological particles.

The uptake of biomass by the solid-phase, measured as a decrease in the optical density of the re-circulated fluid, was followed for 12 h. Distinct kinetic profiles were obtained for each of the materials tested (Fig. 3a,b). Similar to the pulse experi-

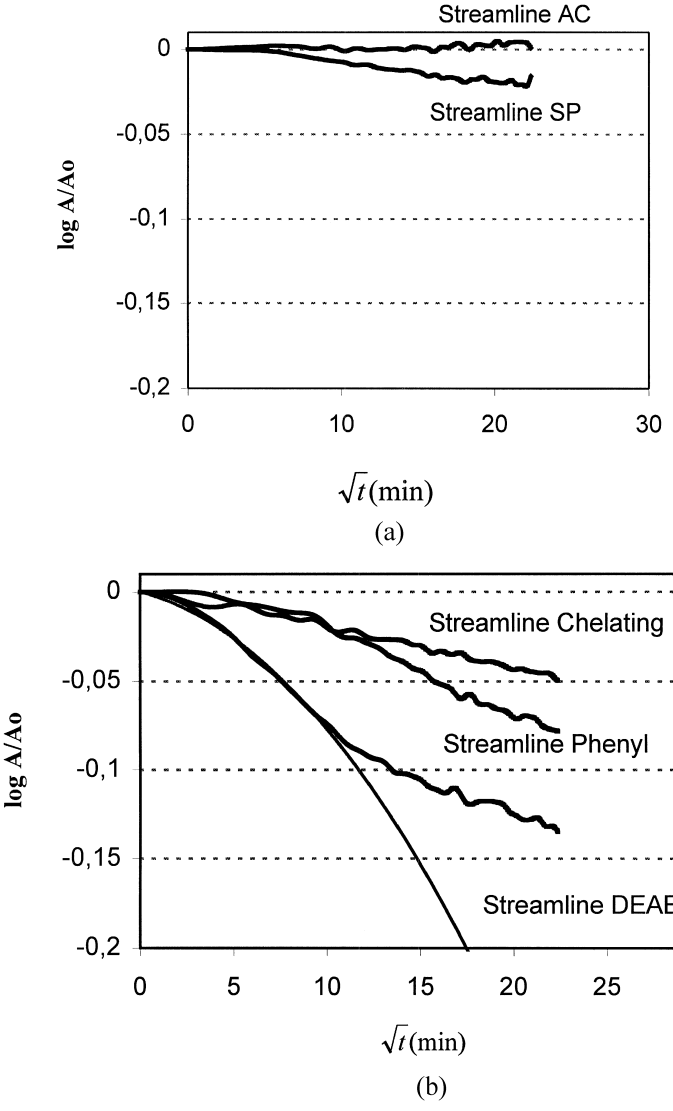


Fig. 3. *S. cerevisiae* adsorption kinetics measured in a stirred vessel ($Re_A = 1500$) showing binding of intact yeast cells (initial cell concentration $4.5 \cdot 10^6$ ml^{-1}) to Streamline adsorbents: (a) Streamline AC and SP; (b) Streamline DEAE, Phenyl and Chelating. Different phosphate based buffers were used for each type of adsorbents as described under materials and methods. The decrease in optical density was continuously monitored up to 12 h by UV absorbance at 600 nm.

ments, the unmodified agarose based support (Streamline AC) showed no biomass adsorption at all probably due to its hydrophilic nature. A similar tendency was observed for the cation-exchanger (Streamline SP) although some biomass build-up was observed at very long contact times, a situation which seems not to be relevant for practical use (Fig. 3a). On the other hand, the anion-exchanger (DEAE Streamline) showed a clear cell uptake. This may be divided in two steps: (a) at low contact times (<3 h) coverage is low and cells binds to the adsorbent by electrostatic interactions (adsorption), and (b) at longer contact times biomass continues to accumulate on the solid surface but with lower rates (layering and adhesion). A description of the kinetics of the diffusion-controlled adsorption of micro-organisms to ion-exchangers has been already proposed [18]:

$$\log\left(\frac{A}{A_0}\right) = kt + k'\sqrt{t}$$
$$k = -4\pi\beta_0 RND \qquad (3)$$
$$k' \geq 2kR/\sqrt{\pi D}$$

where A is the optical density, β_0 is an empirical constant relating cell number with the former, N is the number of adsorbent beads of radius R, D is a coefficient of diffusion, and t is time. This expression is well suited to describe the early period of cell capture. It also clearly visualises the transition between the two phases (adsorption + adhesion) in the case of yeast cells binding to Streamline DEAE (Fig. 3b). The experimental data from the primary cell adsorption phase were successfully fit by the expression:

$$\log A/A_0 = -6.8\cdot 10^{-4}\cdot t - 1.1\cdot 10^{-3}\cdot t^{1/2}$$

When Streamline Chelating (Cu^{2+}) and Streamline Phenyl were evaluated a small contribution during the adsorption phase was found while at long contact times a significant amount of cells was deposited on the stationary phase due to secondary adhesion (Fig. 3b). It should be pointed out that the surface of many cell types is capable of taking part in hydrophobic interactions with ligands and other biological particles or surfaces. Protein and lipids on the cell surface may present hydrophobic domains by adopting particular conformations and orientation. Higher salt concentration in the fluid phase can also induce aggregation due to a decreased electrostatic stabilisation with concomitant cell–cell interaction mediated by Van der Waals attractive forces.

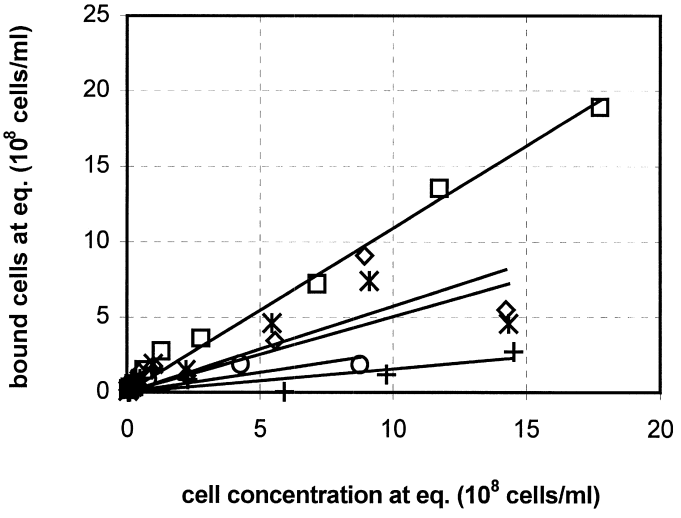
Based on the experimental data obtained here the following series of interaction between intact yeast cells and adsorptive ligands is proposed:

$$\text{DEAE} > \text{PHE} \cong \text{CHE} > \text{SP}$$

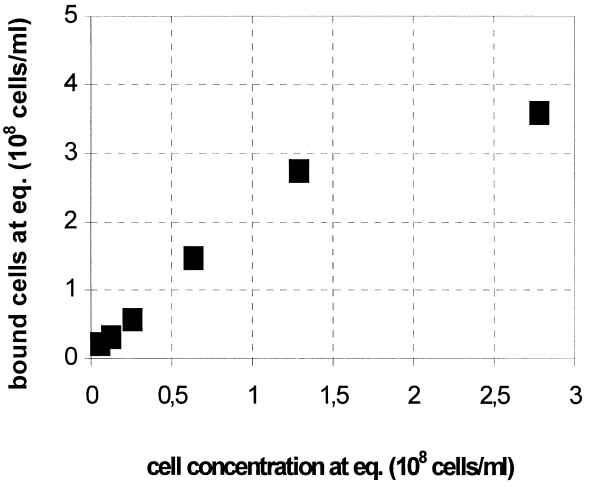
As a consequence, a deteriorated performance of anion-exchange adsorption in expanded beds starting from yeast cell suspension may be anticipated. Furthermore, Phenyl and Chelating materials may require adjustments in operational parameters in order to minimise cell–adsorbent interactions, especially at long contact times or increased biomass load.

3.2. Adsorption isotherm

The interaction between yeast cells and the five stationary phases was quantified in terms of an adsorption isotherm. Contrary to the pulse/response experiment, where low cell concentrations (≅0.1% wet mass) were contacted only for a short time with the expanded adsorbents (contact time $L/U = 144$ s), in these experiments the binding equilibrium was investigated. The solid-phase was not only contacted with the biomass for a long period of time but also challenged by high biomass loads (up to 15% wet weight). The adsorption isotherms for the systems *S. cerevisiae* and each of the Streamline adsorbents under study are shown in Fig. 4. From this plot a linear relationship can be established, in each case, between the amount of cells adsorbed and the equilibrium cell concentration in the liquid. For example, in concentrated biomass suspensions, which are relevant in industrial practice, one may predict the binding of 30–60 g of *S. cerevisiae* cells per l DEAE gel, 15–20 g cells per l Chelating (or Phenyl) gel, and 5–6 g cells per l SP gel. However, in the particular case of Streamline DEAE, two different regions of cell adsorption to the stationary phase can be identified (Fig. 4b). In the low cell concentration area up to $3\cdot 10^8$ cells per ml (corresponding to 1–1.5% wet mass) the apparent equilibrium capacity of the anion-exchanger for yeast cells is significantly higher than the apparent capacity of



(a)



(b)

Fig. 4. (a) *S. cerevisiae* cell adsorption isotherms on Streamline adsorbents using cell concentrations up to 15% wet mass; (b) detail at low cell concentration showing a Langmuir-type of behaviour in case of the DEAE adsorbent. (□) Streamline DEAE, (○) Streamline SP, (◇) Streamline Phenyl, (+) Streamline AC, and (∗) Streamline Chelating-Cu^{2+}.

the other matrices. The isotherm is of a saturation type, a trend which was confirmed for other anion-exchange adsorbents as well (not shown). From these data in can be deducted, that at low cell concentration the equilibrium capacity is determined by the attractive electrostatic interactions between the cells and the adsorbent. At high cell concentration the interactions are dominated by adsorption of cells to adsorbent beads, which may be already covered by cells. In this region of cell/cell adsorption the different electrostatic properties of the stationary phases are no longer relevant. This assumption is especially supported by the fact that even Streamline AC and Streamline SP, which appeared to be inert towards yeast cells in the pulse experiment, do bind cells after very long exposure to high yeast con-

centrations. The absolute values measured for the equilibrium capacity of the adsorbents for intact yeast cells are in the same order of magnitude as data reported in literature for systems, where the intentional immobilisation of cells to various particles was investigated [18].

These data confirm the initial characterisation of the four different systems with regard to cell/adsorbent interaction with the pulse response and the finite bath methods. Apparently the anion-exchanger is much more susceptible to yeast cell adsorption and may thus be less reliable during protein adsorption from whole yeast cell suspensions.

3.3. Stability of the expanded bed in yeast cell suspensions

The stability of expanded beds, in the presence of varying concentration of intact yeast cells, was investigated by RTD measurements. The system response to NaBr (or LiCl) pulses was measured by ISE and the signal was translated into a RTD function. This function was then analysed by the PDE model, yielding the three parameters *Pe*, *N*, and φ. The RTD function for Streamline SP as well as for Streamline Phenyl showed moderate changes upon feeding of the EBA system with the biomass suspension, characterised by a decrease in φ from 1 to 0.7. On the other hand, φ values were found to be almost constant in the case of Streamline Chelating irrespective of the cell concentration in the fluid phase whereas Streamline DEAE fluidisation seems to be severely affected by the presence of *S. cerevisiae*. Analysing all of the experimental RTD functions in terms of the PDE model shows, that φ is >0.75 for most materials irrespective of the cell concentration in the feed and close to 1.0 for the cation-exchanger and the Chelating matrix. Concomitantly high *Pe* numbers (>60) were found in all these cases so it may be concluded that bed stability is not compromised under these conditions. When the same analysis was performed for the anion-exchanger Streamline DEAE a significant drop in φ was found (down to 0.4) accompanied by a similarly drastic reduction in *Pe* (down to 20). Fig. 5 summarises this series of experiments by showing the correlation between the fraction of stable expanded bed (φ) and the cell concentration in the feed for the adsorbents studied. These findings correspond well with the observations described above where the anion-exchanger showed much stronger interaction and faster uptake of suspended yeast cells than the other stationary phases. Based on this set of data we conclude that the already described pulse/response

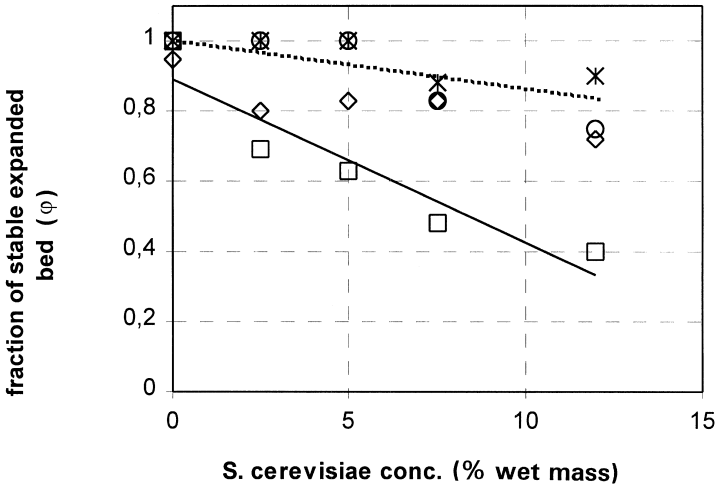


Fig. 5. Correlation between the fraction of stable expanded bed (φ) from residence time distribution analysis (PDE model) and *S. cerevisiae* concentration in the feedstock. Experiments were carried out using different concentration of cells (0, 2.5, 5.0, 7.5 and 12% wet mass) as feedstock and the following Streamline adsorbents: (□) DEAE, (○) SP, (◇) Phenyl, and (∗) Chelating-Cu^{2+}. (Solid line) data trend for interacting system; (dotted line) data trend for low to moderately interacting systems.

and finite bath methods give good indications, which may be used to estimate the stability of different stationary phases when fluidised in particle containing feedstock.

3.4. Breakthrough experiments

Breakthrough curves were measured for the adsorption of BSA to Streamline DEAE and Streamline Phenyl and for Lysozyme on Streamline SP and Streamline Chelating (Cu^{2+}) from particle free feed solutions as well as from feedstock containing *S. cerevisiae* in varying concentration. The curves were analysed in terms of equilibrium capacity as well as of breakthrough capacity at 10% breakthrough. The ratio of breakthrough capacity to equilibrium capacity ($Q_{brk.}/Q_{eq.}$) served as a measure for sorption efficiency. The results of these sets of experiments are summarised in Table 1.

BSA breakthrough in particle free feed solution on Streamline DEAE at $5\cdot10^{-4}$ m s^{-1} and 0.2 m sedimented bed height (contact time $L/U=400$ s) resulted in an equilibrium capacity of 74 kg m^{-3}. The breakthrough capacity at 10% breakthrough was 49 kg m^{-3} yielding a sorption efficiency of 0.66. When a yeast cell suspension was used as a feedstock two different effects were observed: the equilibrium capacity dropped continuously to a value of only 63 kg m^{-3} at 12% wet mass of particle load in the feed. Additionally the sorption efficiency was reduced as well to 0.47 at 5% and furthermore to 0.34 at 12% solids content in the feed. The reduction in equilibrium capacity can be explained as follows: The significant adsorption of cells to the stationary phase may reduce the available number of ligands for protein binding thus reducing the equilibrium capacity for the protein. Additionally it has to be taken into consideration that the RTD analysis showed the existence of stagnant zones (up to 60% of the total column volume as expressed by a φ value of 0.4 at 12% yeast cell concentration in the feed (Fig. 5). In these stagnant zones the accessibility of the adsorbent particles for protein binding definitely is restricted thus contributing to the reduction in equilibrium capacity for the target protein. Sorption efficiency is hampered as well by the existence of stagnant zones. The RTD within the expanded bed significantly deviates from the assumption of plug flow through a perfectly classified

Table 1
Results of BSA adsorption to Streamline DEAE and to Streamline Phenyl and of HEWL adsorption to Streamline SP and to Streamline Chelating (Cu^{2+}) in the absence and presence of *S. cerevisiae* cells ($L=0.2$ m, C_0 BSA 1.5 or 4.2 kg m^{-3}, C_0 HEWL 2.0 or 3.0 kg m^{-3}, $U=5.3\cdot10^{-4}$ m s^{-1})

	Q_{eq} (kg m^{-3})	Q_{brk} (kg m^{-3})	Q_{brk}/Q_{eq}
BSA on Streamline DEAE			
Cell free solution	74	49	0.66
5% cell concentration	71.6	33.5	0.47
12% cell concentration	63	21.2	0.34
Lysozyme on Streamline SP			
Cell free solution	126	115	0.91
2.5% cell concentration	137	125	0.91
12% cell concentration	124	76	0.61
BSA on Streamline Phenyl			
Cell free solution	30.3	19.6	0.64
2.5% cell concentration	29.9	19.6	0.65
10% cell concentration	29.7	15.7	0.53
Lysozyme on Streamline Chelating (IDA-Cu^{2+})			
Cell free solution	84.1	68.6	0.82
2.5% cell concentration	74.3	60.1	0.82
10% cell concentration	68.9	54.9	0.79

and stable bed. Fluid flow is characterised by increased axial mixing and flow channels with an uneven distribution of residence times and this affects the driving force for protein transport to and within the adsorbent particles. Thus the overall efficiency of the bed deviates from an ideal situation. All these effects may be attributed to the strong interaction of yeast cells with the fluidised anion-exchanger as detected and discussed above.

In the case of lysozyme adsorption to Streamline SP breakthrough in particle free feed solution was comparable to breakthrough from a suspension containing 2.5% yeast cells. When 12% yeast cells were included into the feed the equilibrium capacity still was the same, thus demonstrating the existence of a stable expanded bed with particulate fluidisation. The sorption efficiency, however, was reduced to quite some extent. This decrease in efficiency was accompanied by a significant increase in the bed void fraction (from 0.72 in cell free solution and 2.5% yeast suspension to 0.79 in 12% yeast suspension). This increased void fraction may lead to a reduced fluid side transport efficiency which in turn results in earlier breakthrough. The three breakthrough curves are shown in Fig. 6 as C/C_0 vs. time. The almost identical curves obtained at 0% and 2.5% biomass load highlight the conserved performance of the expanded beds while the reduced efficiency at high biomass load is expressed by the significantly earlier breakthrough of the target protein. These experimental findings are in accordance with the observation of only minimal cell–adsorbent interaction and ideal RTD of expanded beds of this stationary phase. Apparently the presence of *S. cerevisiae* does not hamper the stability of expanded beds of Streamline SP and thus the sorption performance of the stable bed is mainly limited by transport processes.

Both Streamline Phenyl and Streamline Chelating (as adsorbents for BSA and Lysozyme, respectively) showed a similar trend, i.e. a slightly decreasing tendency for Q_{eq} (from 30.3 to 29.7 for the Phenyl and from 84.1 to 68.9 for the Chelating materials). Efficiency was hampered only to a limited extent at the higher biomass concentration. These results can be explained considering a higher selectivity of the adsorbents towards the model protein than for the biological solid matter in the feedstock, which led to only minor deviations of bed stability and RTD from ideal cell free solutions. It should be pointed out, however, that the Chelating material seems to be (under similar experimental conditions) of better performance than the Phenyl adsorbent.

4. Conclusions

The efficiency of EBA of proteins from complex biotechnological feedstock has long been known to

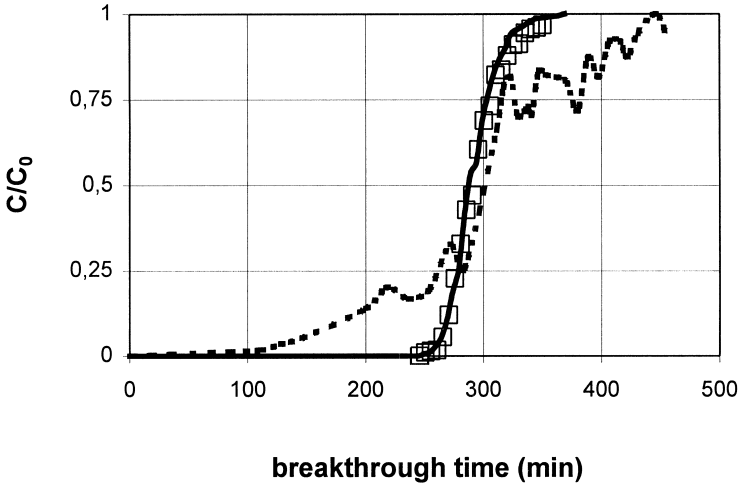


Fig. 6. Breakthrough of HEWL (3 kg m^{-3}) on Streamline SP ($L=0.2$ m, $U=5.3\cdot10^{-4}$ m s^{-1}) at varying *S. cerevisiae* concentration in the feedstock: (□) cell free solution, (solid line) 2.5% wet mass yeast suspension, and (dotted line) 12% wet mass yeast suspension.

be influenced by potential interference of cells or cell homogenates with the stability of the expanded bed, although this was never studied quantitatively. In previous papers we have described two methods, which may be employed for quantification of this interference. In this contribution the correlation between cell–adsorbent interaction, bed stability, and sorption performance is established.

In initial experiments it was shown that adsorption of yeast cells to the anion-exchanger is fast and significant while the cation-exchanger is much less prone to cell–adsorbent interactions particularly at low solid content. Group affinity adsorbents were found to be less sensitive to the presence of biomass although they can be substantially challenged at high biomass load and at long contact times. Cell attachment apparently was dominated by attractive electrostatic interactions during the primary phase. These findings were confirmed by a pulse response technique, kinetic analysis, as well as by adsorption isotherms. Severe cell-adsorbent interactions directly led to a reduced stability of the expanded bed, a fact which could be quantified by analysing the RTDs by the PDE model. The most significant parameter of this model is the fraction of well fluidised bed (φ), which fulfils the criterion of particulate fluidisation and stable expansion. The non interacting system cation-exchanger/yeast was characterised by a value of φ close to 1, while the strongly interacting system anion-exchanger/yeast showed steadily decreasing φ with increasing load of biomass. An intermediate situation was found for Phenyl and Chelating adsorbents. RTDs characterised by decreasing φ indicate the presence of flow channels and stagnant zones within the bed, which in turn is expected to give deteriorated sorption performance. This assumption could be verified by breakthrough experiments. Here non interacting systems demonstrated conserved equilibrium capacity and sorption performance.

Deteriorated sorption performance has already been discussed in literature as a function of increased axial mixing or system stagnation [3,11,19]. In this paper a direct correlation between the presence of cells in real feedstock and bed stability on one hand and efficiency of adsorptive purification on the other hand was demonstrated. This underlines the importance of incorporating investigations on cell–adsorbent interaction into process design in EBA. It is strongly recommended to optimise operating conditions in EBA not only with regard to maximum protein binding capacity but also regarding minimised cell–adsorbent interaction. The four methods used here may be employed by potential users of EBA for their respective experimental system. The following strategy is proposed:

1. Qualitative test of cell–adsorbent interaction by the pulse response method and/or kinetic experiments.
2. Optimisation of conditions in the feed (pH, ionic strength, additives) in order to allow full transmission (>95%) of cells through the expanded bed or decreased uptake in the batch mode.
3. Measurement of RTD during fluidisation of the stationary phase in real particle containing feedstock.
4. Optimisation of particle load to the expanded bed in order to allow optimum bed stability under real broth conditions (borderline criterion: $\varphi > 0.8$, $Pe > 40$).

EBA processes optimised under these conditions will then show conserved sorption performance compared to adsorption from clarified supernatant. Neglecting a potential influence of cell–absorbent interactions, however, may cause disappointing deviations between the performance of EBA estimated from ideal supernatant and the results obtained from whole broth.

5. Symbols and abbreviations

A	UV absorbance
BSA	bovine serum albumin
C_{cells0}	initial cell concentration, cells ml^{-1}
C_{cellseq}	cell concentration at equilibrium, cells ml^{-1}
C_0	initial protein concentration, kg m^{-3}
D	diffusion coefficient, $\text{m}^2\ \text{s}^{-1}$
d_p	particle diameter, m
d_{pmin}	smallest particle diameter within a distribution, m
d_{pmax}	largest particle diameter within a distribution, m
EBA	expanded bed adsorption
h	column length, m

HEWL	hen egg white lysozyme
ISE	ion selective electrode
L	sedimented bed length, m
N	mass transfer parameter in PDE model, (–)
N_R	stirrer speed, s^{-1}
PDE	plug flow with dispersion and mass exchange with stagnant zones
Pe	Peclet number, (−)
Q	adsorption capacity for *S. cerevisiae*, cells ml^{-1}
$Q_{brk.}$	protein breakthrough capacity at 10% breakthrough, kg m^{-3}
$Q_{eq.}$	protein equilibrium capacity, kg m^{-3}
R	adsorbent particle radius, m
Re_A	modified Reynolds number; $d_p^2 \rho N_R / \mu$, (−)
t	time, s
U	fluid velocity, m s^{-1}
V_l	fluid phase volume, m^3
V_s	stationary phase volume, m^3
μ	fluid viscosity, Pa s
ρ	fluid density, kg m^{-3}
φ	fraction of stable expanded bed, (−)
$\phi_{int.}$	internal diameter of column, m

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References

[1] R. Hjorth, P. Leijon, A.K. Barnfield-Frej, C. Jägersten, in: G. Subramanian (Ed.), Bioseparation and Bioprocessing, Vol. 1, Wiley-VCH, Weinheim, 1998.
[2] J. Thömmes, Adv. Biochem. Eng. 58 (1997) 185.
[3] Y.K. Chang, H.A. Chase, Biotechnol. Bioeng. 49 (1996) 512.
[4] R. Hjorth, S. Kämpe, M. Carlsson, Bioseparation 5 (1995) 217.
[5] A. Karau, C. Benken, J. Thömmes, M.-R. Kula, Biotechnol. Bioeng. 55 (1997) 54.
[6] N.M. Draeger, H.A. Chase, Bioseparation 2 (1991) 67.
[7] H.A. Chase, N.M. Draeger, Sep. Sci. Technol. 27 (1992) 2021.
[8] K.B. Barnfield-Frej, R. Hjorth, A. Hammarstrom, Biotechnol. Bioeng. 44 (1994) 922.
[9] J.C. Erickson, J.D. Finch, D.C. Greene, in: B. Griffiths, R.E. Spier, W. Berthold (Eds.), Animal Cell Technology: Products for Today, Prospects for Tomorrow, Butterworth & Heinemann, Oxford, 1994, p. 557.
[10] J. Feuser, J. Walter, M.-R. Kula, J. Thömmes, Bioseparation 8 (1999) 99.
[11] H.M. Fernández-Lahore, R. Kleef, M.-R. Kula, J. Thömmes, Biotechol. Bioeng. 64 (1999) 484.
[12] J. Villermaux, W.P.M. van Swaaij, Chem. Eng. Sci. 24 (1969) 1097.
[13] S.Y. Suen, M.R. Etzel, Chem. Eng. Sci. 47 (1992) 1355.
[14] M.M. Bradford, J. Anal. Biochem. 72 (1976) 248.
[15] S. Yamamoto, Y. Sano, J. Chromatogr. 597 (1992) 173.
[16] D.F. Evans, H. Wennerström, The Colloidal Domain, VCH, Weinheim, 1994.
[17] M. James, in: N. Mozes, P.S. Handley, H.J. Busscher, P.G. Rouxhet (Eds.), Microbial Cell Surface Analysis, VCH, Weinheim, 1991, p. 220.
[18] S.L. Daniels, in: G. Bitton, K.C. Marshall (Eds.), Adorption of Microorganisms to Surfaces, Wiley, New York, 1980, p. 7.
[19] J. Thömmes, M. Weiher, A. Karau, M.R. Kula, Biotechnol. Bioeng 48 (1995).