cBSA-147 for the preparation of bacterial biofilms in a microchannel reactor[a)]

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Whole cells are attractive biocatalysts, particularly if the reaction requires cofactors or involves multiple transformations. Immobilization of the catalyst is often a prerequisite for continuous processes. The highly cationic chemically modified plasma protein bovine serum albumin (cBSA-147) has been applied for the electrostatically mediated immobilization of the planktonic bacterium *E. coli BL21 star (DE3)*, and the resulting biofilms were superior to those formed on poly-L-lysine coated surfaces. The biocatalyst was immobilized in a capillary column (inside diameter of 530 μm and $L=30$ m) and evaluated in the enantioselective reduction of ethyl acetoacetate to *R*-(-)ethyl hydroxybutyrate. In continuous operation in the microreactor format, the productivity of the cells was about 30% higher than that determined in a bench-scale fermentation system. This increase is attributed to the improved mass transfer over short geometrical dimensions. The similarity in the results indicates that studies on a biofilm-coated microreactor can be used for the accelerated collection of data for process optimization. © *2010 American Vacuum Society.* [DOI: 10.1116/1.3474475]

I. INTRODUCTION

The application of biocatalysts for the synthesis of pharmaceutical intermediates or drug molecules represents an emerging area since remarkable chemo-, regio-, and stereoselectivity can be achieved. Biocatalytic processes are often considered more environmentally friendly than chemical approaches involving transition metal catalysts and organic solvents. To fully utilize the intrinsic advantages of biocatalysts, the development of techniques that promote better integration of biocatalysis into industrial production of bulk commodities and fine chemicals is of growing importance.[1–3]

Microreactors are particularly attractive for multiphase reactions. The small diameter in the order of 100 μm –1 mm yields efficient mass and heat transfer due to the large surface-to-volume ratio and allows for precise control of the reaction variables thus facilitating parallel rapid screening of reactions with minimal amounts of reagents.[4] Small volumes are a key concern for combinatorial synthesis which is always performed on a picomolar scale. If larger product quantities are required, the number of reactors can simply be increased, the so-called scale out principle.[5] In recent years, microchannel reactors have been extensively used in analysis and synthesis[6] as well as for the accelerated collection of data for the optimization of biocatalytic processes.[1,2] In this way, the speed of the bioprocess design can be improved and a wide range of variables can be examined in a short time.

Microenzyme-reactors have been described, where the enzymes were either in solution or in their immobilized form. In solution, the trypsin catalyzed hydrolysis of benzoyl-arginine-p-nitroanilide[7] and the glycosidase-catalyzed hydrolysis[8] showed three to five times higher reaction yields in a continuous flow microreactor. These results confirm the excellent mass transfer in microchannel devices leading to improved chemical yields.

Whole cells offer distinct advantages as biocatalysts, particularly if complex transformations involving cofactors are considered. The successful immobilization of a variety of cells in different matrices such as alginate, carrageenan, or polyacrylamide gels has been reported.[9–11] Immobilization of whole cells is generally more sensitive compared with isolated enzymes since cell viability is crucial to maintain high activity of the cells' intrinsic enzymes. Recently, the use of calcium alginate as an immobilization matrix for the recombinant *E. coli* whole cell biocatalyst for the enantioselective reduction of ethyl acetoacetate (EAA) to *R*-(-)ethyl hydroxybutyrate (EHB) has been described.[12] In this system, a productivity of 1.4 g_{EHB} per gram of wet cell weight (g_{WCW}) per hour with a space time yield of 600 g_{EHB}/l day has been demonstrated. In the present work, the immobilization of these recombinant *E. coli* cells inside a fused silica capillary for application in a microreactor format is explored.

Bacterial cell walls are generally negatively charged due to the presence of lipopolysaccharides. Electrostatic interactions offer a fast, mild, and easy to implement approach for the immobilization of whole cells onto the wall of a

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microreactor.[13] In contrast to the covalent attachment of cells, the attachment via noncovalent interactions offers less synthetic steps to chemically modify the glass surface and to connect the surface and the cells. In this way, no reactive reagents are needed, which can also decrease cell viability. The immobilization of several strains of *E. coli* with poly-L-lysine (PLL) as adhesion promotor via electrostatic interactions has been reported.[14] However, the application of PLL was also limited by its cytotoxicity. In cBSA-147, the acid functionalities of the glutamic and aspartic acids present in native bovine serum albumin have been converted into amino groups by reaction with ethylenediamine.[15–17] This converts the surface charge of bovine serum albumin (BSA) from anionic to cationic at physiological *p*H. Glass surfaces which have been incubated with cBSA-147 assume a positive charge, and therefore can aid in immobilizing bacterial cells. In the following, the potential of the novel biocoating cBSA-147 for monolayer formation on glass surfaces in a microchannel reactor as well as for the immobilization of cells for the production of EHB will be explored.

II. MATERIALS AND METHODS

A. Rhodamine-labeled bovine serum albumin (BSA-Rho)

1 ml of a freshly prepared aqueous solution of tris(2-carboxyethyl)-phosphine hydrochloride (1 mM, 1 μmol) was added to BSA fraction V (Sigma) (300 mg, 4.5 μmol) in phosphate buffer (300 ml, 50 mM, *p*H 7.4) and the reaction mixture was stirred for 10 min at room temperature. A solution of tetramethylrhodamine-5-maleimide (4.3 mg) in DMSO (1 ml) was added and the reaction mixture was stirred at room temperature for 1 h under argon atmosphere. Excess dye was removed by ultrafiltration and size-exclusion chromatography (BioGel P 30, milli-Q water) and the recovered protein was lyophilized.

B. Cationized bovine serum albumin (cBSA-147)

Native BSA contains about 40 aspartic acid, 59 glutamic acid, 59 lysine, and 23 arginine residues.[18] Since there are more negatively charged amino acid residues than basic residues, BSA carries a net negative charge at physiological *p*H.[19] The procedure for achieving the highly cationic protein-polyelectrolyte has been reported before.[16,17] Ethylenediamine (EDA) (10 mmol) was dissolved in 5 ml milli-Q water and the *p*H of the solution was adjusted to 4.75 with 6*N* HCl. Rho-labeled or unlabeled bovine serum albumin (0.75 μmol, 1 eq.) was dissolved in 5 ml milli-Q water and added to the EDA solution. 1-ethyl-3-(3′-dimethylamino-propyl)-carbodiimide hydrochloride (94 μmol, 125 eq.) was added to the mixture and stirred for 2 h at room temperature while the *p*H was maintained at 4.75. The cationization reaction was stopped by adding 1 ml 4M acetate buffer (*p*H 4.75). Low molecular weight species were removed by repetitive ultrafiltration (Vivaspin 20, 30 kDa MWCO). The concentrated protein was dialyzed against milli-Q water and lyophilized.

C. Mass spectrometry (matrix-assisted laser desorption ionization time-of-flight)

Mass spectra were recorded using a Bruker Autoflex II matrix-assisted laser desorption ionization time-of-flight instrument. Sinapinic acid (saturated sinapinic acid in 0.1% trifluoroacetic acid: acetonitrile 2:1) was applied as sample matrix. The average molecular weight of the protein increased from 66.9 kDa for the rhodamine labeled native BSA to about 70.6 kDa for rhodamine labeled cBSA-147, indicating the conversion of about 87 of the 99 carboxylic acid groups of glutamate and aspartate residues into primary amino groups.

D. Pretreatment of the surface of a capillary

The pretreatment procedure for glass surfaces has been described.[20] A polyamide coated quartz capillary [Polymicro Technologies, Phoenix, Az, USA; inside diameter (ID) of 0.53 mm and $L=30$ m) was filled with 1*N* NaOH and kept in an oven at 90 °C for half an hour. The capillary was rinsed thoroughly with milli-Q water to neutral *p*H, filled with 1*N* HCl and allowed to stand for 10 min at room temperature. The capillary was again rinsed with milli-Q water to neutral *p*H and kept filled with milli-Q water until used.

E. Preparation of samples for microscopic characterization

20 μl of a 10 μM cBSA-147 solution was dropped onto a pretreated Teflon printed glass slide with five holes of 8 mm diameter (Electron Microscopy Sciences) and incubated for 10 min. The droplet was pipetted off and the slide was rinsed carefully with milli-Q water. Thereafter, the slide was left to dry in a laminar flow hood. Then, 20 μl of an *E. coli* cell suspension (4 mg/ml) were added. After 10 min, the supernatant cell suspension was pipetted off and the slide was again rinsed carefully with milli-Q water. Viability of the cells was examined by incubating the cells with fluorescein diacetate.

F. Microscopic Imaging

Fluorescence images were taken using an Olympus microscope BX 51. For axially resolved experiment, an Olympus FV300 inverted confocal microscope was used.

G. Atomic force microscopy

Measurements were performed on a VEECO dimension 3100 nanoscope IV using the tapping mode. A tip with a force constant of 40 N/m operated at a resonant frequency of 300 kHz was used.

H. Quartz crystal microbalance

The adsorption kinetics of cBSA-147 on a glass surface has been studied by means of a quartz crystal microbalance (QCM). A Q-Sense E1 model (Q-Sense, Gothenburg, Sweden) with AT-cut quartz crystals (QSX 303) with a fundamental resonant frequency of 5 MHz was used. One side of

each crystal was coated by the manufacturer with 50 nm of SiO_2. The crystals were cleaned by immersion in a 5:1:1 mixture of milli-Q water, ammonia (25% v/v), and hydrogen peroxide (30% v/v) for 10 min at 75 °C, followed by thorough rinsing with milli-Q water and drying with a dry nitrogen gas stream. The crystals were subsequently treated twice with UV light and ozone for 10 min, and each time rinsed with ultrapure water to remove organic contamination. The freshly cleaned quartz crystal was directly mounted in a flow cell with the silicon oxide surface exposed to the solution. All measurements reported in this article were done with the system temperature stabilized at 23 ± 0.5 °C. First the sample cell was filled with milli-Q water to equilibrate the system for about 15 min. For adsorption of cBSA-147 onto the bare surface, 1 ml of the protein sample solutions (10 μg/ml) was pumped through the flow cell by a peristaltic pump at a flow rate of 250 μl/min. The solution was incubated on the surface for approximately 30 min. To check if the adsorption is complete, another 500 ml of protein solution was added to the crystal and incubated for another 35 min. Afterward the surface was strongly rinsed with ultrapure water at a flow rate of 1 ml/min for 2 min. The kinetics of sample adsorption and desorption were followed by changes in the resonant frequency and the dissipation of the crystal vibrations. Frequency and dissipation changes were recorded simultaneously at different overtones [$n=3$ (15 MHz), 5 (25 MHz), 7 (35 MHz), 9 (45 MHz), and 11 (55 MHz)].

I. Contact angle measurement

Contact angles were determined by the static sessile drop method on a DFTA 1000 (First Ten Angstroms Inc.). The images were analyzed with software FTA32. Distilled water was used as probe liquid.

J. Transformation of *E. coli* with plasmids bearing the gene encoding for *Lb*ADH and cell cultivation

Plasmid pBtac-*Lb*ADH (a gift from the Institute for Biotechnology, Research Center Julich, Germany), carrying the *adh* and *bla* genes for resistance against ampicillin, was transfected into *E. coli* BL21 Star (DE3) (Invitrogen, Singapore). 5 μl of plasmid solution were added to an Eppendorf tube containing *E. coli* and the cells were incubated for 20 min at 4 °C to induce plasmid uptake. The cells were kept in a 42 °C water bath for 90 s to inactivate the nucleases, cooled to 4 °C for 5 min and 400 μl of SOC medium were added. They were then incubated at 37 °C for 1 h with light shaking. 25 μl of cell suspension was streaked on LB agar plates containing 100 mg/l of ampicillin and the plates were incubated overnight at 37 °C.

A colony of transformed *E. coli* was selected randomly from a LB agar plate and inoculated in 100 ml LB medium with 100 mg/l ampicillin in a 250 ml shake flask. The preculture was incubated overnight at 30 °C and shaken at 150 rpm. 500 μl of the preculture was then transferred into 500 ml TB medium with 200 mg/l ampicillin in a 1 l Nalgene culture flask, and this main culture was incubated at 37 °C for 24 h. The cells were harvested and stored in 50 mM phosphate buffer *p*H 6.

K. Preparation of the microchannel reactor

1. Coating of cBSA-147

A polyamide coated quartz capillary (Polymicro Technologies, Phoenix, Az, USA; ID of 0.53 mm and $L=30$ m) was used as microreactor. cBSA-147-Rho (10 ml, 10 μM) was circulated through the capillary at a flow rate of 0.16 ml/min. Circulation of this solution was continued for about 3 h, followed by rinsing with 50 mM phosphate buffer (*p*H 6). As comparison experiment, poly-L-lysine [Sigma P8920, 0.1% (w/v)] was used with the same coating procedure.

2. Biofilm formation with E. coli BL21 star (DE3) cells

25 ml of an *E. coli* BL21 star (DE3) cell suspension with a concentration of 4 mg/ml was circulated through the capillary at a flow rate of 0.16 ml/min. The optical density of the cell suspension was monitored at 660 nm. Circulation of the cell suspension was continued for about 4–5 h until no further decrease in the optical density was detected. Subsequently, the coated capillary was rinsed with 50 mM phosphate buffer (*p*H 6) and the cell number in the biofilm was determined from the optical density of the remaining cell suspension.

L. Biotransformation of EAA in the microchannel reactor

The coated capillary was immersed in a thermostated water bath to maintain the reaction temperature at 37 °C. A solution (25 mM EAA, 50 mM isopropanol, and 2 mM Mg^{2+} in 50 mM phosphate buffer *p*H 6) was pumped through the microreactor. The solution exiting the reactor was collected and extracted twice with diethyl ether; the ethereal phase was dried over Na_2SO_4 and concentrated in vacuum. Thereafter, conversion was determined by GC on an HP5 column and the optical purity was quantified with a chiral column Supelco BetaDex 325.

III. RESULTS AND DISCUSSION

A. Formation of cBSA-147 monolayers on glass surfaces

Cationized bovine serum albumin (cBSA-147) is characterized by a high density of primary amino groups, which are positively charged at a *p*H below 9. Due to its net positive charge, cBSA-147 readily adsorbs on glass surfaces. The formation of cBSA-147 monolayers was investigated via contact angle measurements as well as Atomic Force Microscopy (AFM). The contact angle between a water droplet and the cBSA-coated surface was $61 \pm 2°$ compared to $30 \pm 2°$ on the blank glass slide, indicating the presence of a hydrophobic layer on the glass surface after exposure to cBSA-147. Figure 1 shows an AFM image of a glass slide before and after coating with cBSA-147. Whereas the original glass

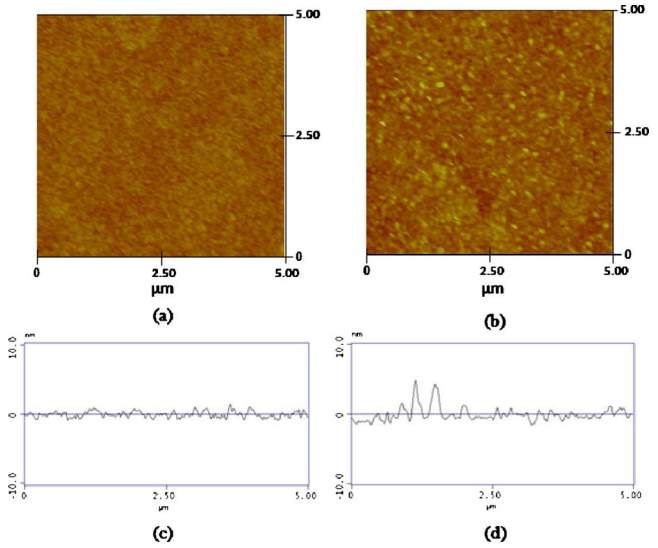

FIG. 1. (Color online) AFM images of the glass surface after (a) no pretreatment (b) incubation with cBSA-147 for 10 min. Line profile section for (c) blank glass surface (d) after incubation with cBSA-147 for 10 min.

surface was essentially smooth, features of about 5–6 nm high were detected after incubation with the cBSA-147 solution. This height agrees well with the diameter of the BSA molecule. However, the lateral width of the features was much wider (about 200 nm), perhaps indicating the adsorption of cluster or raft of cBSA-147 at the surface.

To confirm the formation of a homogeneous albumin layer, cBSA-147 labeled with a rhodamine dye was coated on a glass slide and investigated under the fluorescence microscope. The observation of a uniform red emission from the entire coated surface indicates that within the resolution of the optical microscope, cBSA-147 was evenly distributed over the entire surface (figure not shown).

Monolayer formation of cBSA-147 on a SiO_2 surface was further confirmed by QCM measurements. The result shown in Fig. 2 reveals that cBSA-147 adsorbs on the glass surface within the first 2 min of incubation. After incubation for 30 min, fresh cBSA-147 solution was added, but no further change in frequency was observed indicating that the surface was already completely covered with cBSA-147. Heavy rinsing with milli-Q water did not yield any frequency shift.

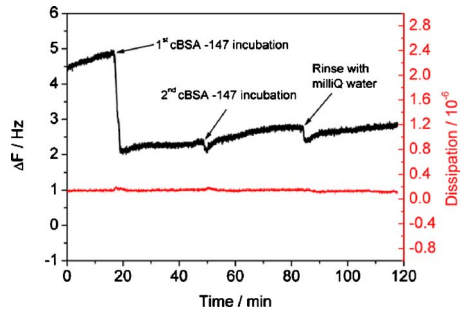

FIG. 2. (Color online) QCM measurement of the adsorption kinetics of cBSA-147 on bare glass. Upper line and scale on the left correspond to the frequency shift and bottom line and scale on the right show the dissipation.

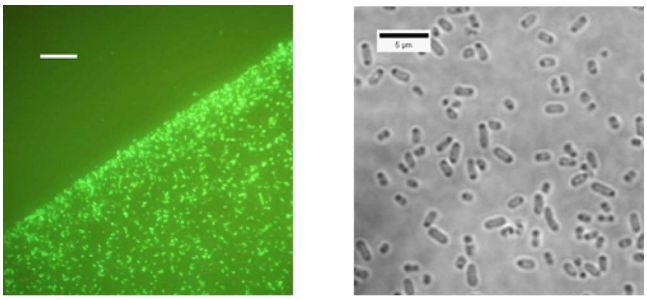

FIG. 3. (Color online) (a) Cells adhering electrostatically on the cBSA-147 coated part of a glass slide (bottom) whereas no cells were found on the glass surface without coating (fluorescence image after incubation with fluorescein diacetate, scale bar: 20 μm). Fluorescence indicates live cells. (b) Immobilized cells at higher magnification (scale bar: 5 μm).

Apparently, cBSA-147 was strongly bound to the glass surface. The low dissipation values of maximum 0.2×10^{-6} further indicate the formation of a stiff and densely packed monolayer where no additional material was loosely bound on top of the first layer. These results are in agreement with recent data.[21]

B. Formation of *E. coli* monolayers

After coating of the glass surface with cBSA-147, the adsorption of bacterial cells as second layer was investigated. The driving force of the adsorption is the strong electrostatic interaction between positively charged proteins and negatively charged cell walls of the bacteria cells. Figure 3(a) depicts the adsorption of *E. coli* cells onto a glass surface treated with cBSA-147 compared to a surface without cBSA-147 coating. No cells attached to the glass slide in the absence of cBSA-147 (shown in the top half of the micrograph). However, in the presence of cBSA-147, a uniform distribution of *E. coli* cells over the entire field was found. Thereafter, the cells were incubated with fluorescein diacetate and the pronounced green fluorescence indicates a high number of viable cells on the surface. The adsorption of the bacterial cells was further investigated under the confocal microscope [Fig. 3(b)]. A z-scan, where a depth of 8 μm was recorded with steps of 0.2 μm revealing that the *E. coli* cells were solely observed in the layer directly above the glass surface indicating the formation of a cell monolayer.

As comparison, PLL, a well-known adhesion promoter which is readily available, was also investigated. PLL has some limitations such as nonspecific cell adhesion, cytotoxicity, and abnormal cell spreading.[22] *E. coli* cells were immobilized on a glass surface incubated with PLL. However an inhomogeneous cell layer was formed, where the cells adhered in less tightly bound clusters and larger aggregates. In contrast, cBSA-147 facilitated the adsorption of individual bacteria cells, which were uniformly distributed over the entire surface. Typical micrographs are shown in Fig. 4. The cell numbers on glass surfaces preincubated with varying concentrations of cBSA-147 (2–10 μM) was essentially the same (data not shown). This finding agrees well with the

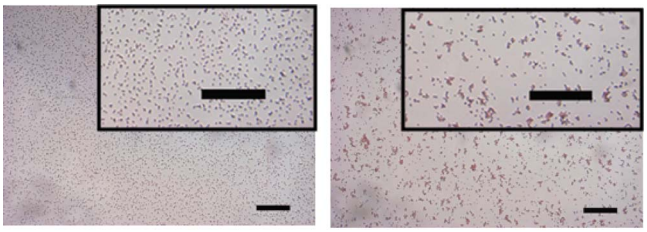

FIG. 4. (Color online) (a) Homogeneous layer of adhering cells on glass surface coated with cBSA-147, and (b) layer of inhomogeneously adhering cells on a glass surface coated with poly-L-lysine. The *E. coli* cells have been stained with safranin O. Scale bar: 50 μm. Insert: image at higher magnification (scale bar 50 μm).

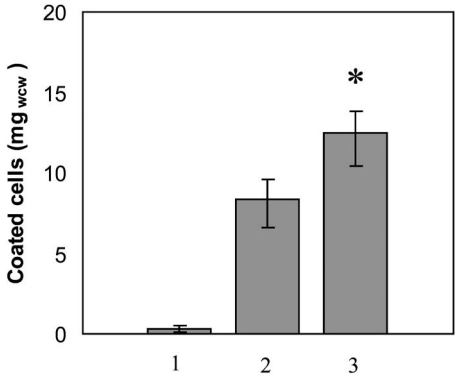

FIG. 6. Biomass of *E. coli* cells immobilized in differently pretreated microchannel reactors; 1-blank glass slide, 2-PLL, 3-cBSA-147. The data were based on three independent experiments and plotted as mean of the measurements and standard deviation as the error bars.

QCM measurements where rapid monolayer formation and coverage of the entire surface even for very dilute solutions was suggested. For all further experiments, a 10 μM cBSA-147 solution was selected. Then, the sequence by which the individual components were added was also investigated. A homogenous cell layer was only obtained if the glass surface was first incubated with cBSA-147 before the cell suspension was added [Fig. 5(c)]. Figure 5(a) clearly shows that no interaction of the *E. coli* cells and the glass surface occurred if no cBSA-147 was applied since only very few cells were detected on the surface. In case the cell suspension and cBSA-147 were mixed before coating, large cell agglomerates were found, as shown in Fig. 5(b). In this case, cBSA-147 attached most likely to the cell walls of different cells thus leading to interactions between the bacterial cells rather than to the desired surface coating.

C. Production of *R*-(-)ethyl hydroxybutyrate in a cBSA-147 coated enzymatic microchannel reactor

Since it has been demonstrated that cBSA-147 efficiently immobilized cells on a glass surface, it was straightforward to investigate the suitability of this novel biocoating for the immobilization of cells in a microchannel reactor. Recombinant *E. coli* overexpressing *Lb*ADH was used as biocatalyst for the bioreduction of EAA to *R*-(-)EHB in a silica capillary microchannel. Both cBSA-147 and PLL were applied independently to fix the cells in the reactor. Figure 6 shows the amount of coated cells in the microchannel, which was determined by monitoring the optical density of the cell concentration during the coating process. Three individual coating experiments were carried out for PLL and for cBSA-147. The efficiency of the cell coating was estimated considering the average dimensions of an *E. coli* cell. cBSA-147 allowed a higher cell density of about 39% of the geometric surface area, whereas in case of PLL, only 26% of the total surface area was covered with the *E. coli* cells.

The bioreduction of ethyl acetoacetate to *R*-(-)ethyl hydroxybutyrate was conducted at the same parameters that were identified above and which should represent an optimum for larger scale experiments. The optimal temperature for recombinant *E. coli* strain BL21 star (DE3) to achieve high bioconversion is about 37 °C. In the experiments with gel-entrapped *E. coli* cells in a plug flow reactor, we have shown that the formation of *R*-(-)ethyl hydroxybutyrate proceeded with >99% enantiomeric excess and a productivity up to 1.4 g_{EHB}/g_{wcw} h was achieved.[12] The flow rate of the reaction mixture through the catalyst coated microchannel constitutes an important parameter to yield maximum mass transfer and to minimize leaching of the catalyst. Therefore, the flow rate through the cBSA-147 coated microreactor was varied in order to identify the impact of this parameter on the productivity of the microreactor. Figure 7 shows that the productivity depends on the flow rate. The highest productivity of 1.9 g_{EHB}/g_{wcw} h was achieved at a flow rate of about 18.5

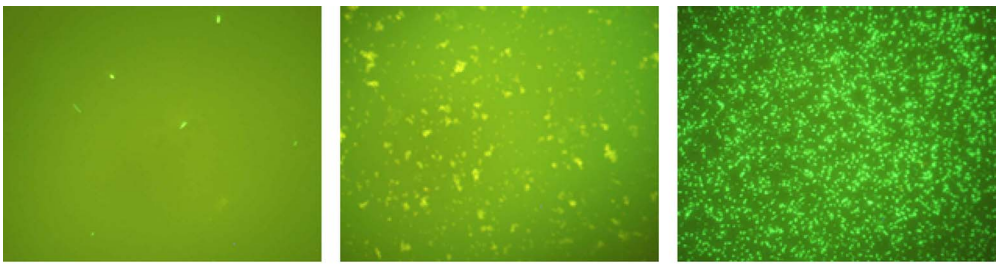

FIG. 5. (Color online) Optimal coating sequence (a) 4 mg/ml cell solution followed by 10 μM of cBSA-147, (b) premixing 4 mg/ml of cell suspension and 10 μM of cBSA-147 before applying to the glass surface, and (c) 10 μM of cBSA-147 followed by 4 mg/ml of cell solution.

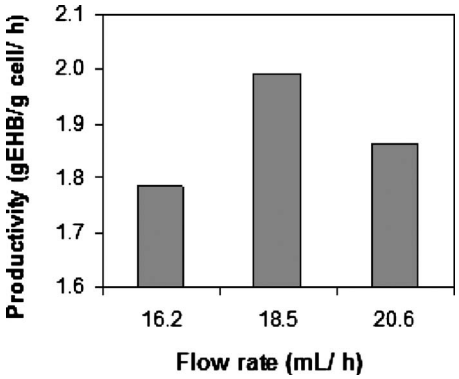

FIG. 7. Productivity of the immobilized cells on a cBSA-147 coated microchannel as function of substrate flow rate. Reaction conditions: 13.5 mg_{wcw}, 25 mM of EAA, 50 mM IPA, 2 mM Mg^{2+}, and 37 °C.

ml/h corresponding to a retention time of about 21.4 min. This is about 30% higher than value measured in a packed bed benchtop fermenter, probably due to the improved mass transfer over the smaller geometrical scale. However, these results are sufficiently close to give us confidence that data on metabolic rates as a function of temperature and medium composition can be directly transferred to a larger scale.

In order to determine the long-time stability of the cells coated by cBSA-147 or PLL in the microchannel, the microreactors were operated continuously. As a control, recombinant *E. coli* cells were applied without any cationic layer via the same coating procedure. As expected, no reaction was observed in the absence of any cationic coating agent since only negligible amounts of cells adhered to the untreated glass surface in the microchannel. Likewise, after pretreatment with native BSA, no bacterial cell adsorption and consequently, no product formation were detected.

Previously, it has been reported that the conversion decreased after about 18 h of continuous operation time in a similar experiment with a reactor packed with *E. coli* cells immobilized in alginate beads.[12] Using the adhesion promoters, a similar lifetime at an even higher productivity of up to 2 g_{EHB}/g_{wcw} was found, which could be maintained for up to 4 days before the productivity gradually decreased (Fig. 8). However, after 4 days under continuous operation, the productivity of the biocatalyst immobilized by PLL decreased faster compared with cBSA-147. In case of cBSA-147, product formation was observed until day 9 under continuous flow conditions.

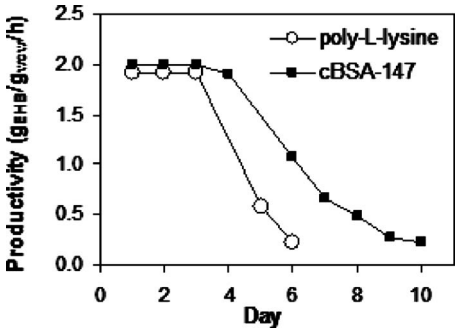

FIG. 8. Sustainability of the coated microreactor (25 mM of EAA, 50 mM IPA, 2 mM Mg^{2+}, and 37 °C). cBSA-147 coated cells: 13.5 mg_{wcw}, substrate solution flow rate 18.5 ml/h; PLL coated cells: 8.4 mg_{wcw}, substrate solution flow rate 18.8 ml/h.

IV. SUMMARY AND CONCLUSIONS

We have demonstrated that the protein-based cationic polyelectrolyte cBSA-147 serves as a biocompatible adhesion promoter for the biofilm formation of recombinant *E. coli* whole cells on glass surfaces. In contrast to alternative coating agents such as PLL, cBSA-147 allowed the formation of homogeneous cell monolayers with significantly increased cell densities. The immobilization process occurred very rapidly and under mild conditions. The monolayer contained mostly viable cells again supporting the high biocompatibility of cBSA-147. The bioreduction of EAA to *R*(-)-EHB in a silica capillary microchannel under continuous process proceeded with up to 30% higher efficiency than that observed in a large-scale packed bed flow reactor. The cells in this wall-coated microchannel reactor showed good sustainability for 3 days without any drop of activity and product formation was maintained at high level for about 9 days. Our results clearly demonstrate that cBSA-147 is well suited for immobilizing a recombinant *E. coli* as biocatalyst since it only marginally affects cell viability and cell function. Due to the cost-efficient and ready availability of the starting material, bovine serum albumin, and the facile and reproducible preparation procedure of cBSA-147 an efficient alternative to commercially available materials such as PLL has been identified that might allow improved productivities and longer reactor process times.

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