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Activity and Lifetime of Urease Immobilized Using Layer-by-Layer Nano Self-Assembly on Silicon Microchannels

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Abstract

Urease has been immobilized and layered onto the walls of manufactured silicon microchannels. Enzyme immobilization was performed using layer-by-layer nano self-assembly. Alternating layers of oppositely charged polyelectrolytes, with enzyme layers "encased" between them, were deposited onto the walls of the silicon microchannels. The polycations used were polyethylenimine (PEI), polydiallyldimethylammonium (PDDA), and polyallylamine (PAH). The polyanions used were polystyrenesulfonate (PSS) and polyvinylsulfate (PVS). The activity of the immobilized enzyme was tested by pumping a 1 g/L urea solution through the microchannels at various flow rates. Effluent concentration was measured using an ultraviolet/visible spectrometer by monitoring the absorbance of a pH sensitive dye. The architecture of PEI/PSS/PEI/urease/PEI with single and multiple layers of enzyme demonstrated superior performance over the PDDA and PAH architectures. The precursor layer of PEI/PSS demonstrably improved the performance of the reactor. Conversion rates of 70% were achieved at a residence time of 26 s, on d 1 of operation, and >50% at 51 s, on d 15 with a six-layer PEI/urease architecture.

Index Entries: Silicon microchannels; urease; architecture; polyelectrolytes; first-order constant.

Introduction

Chemical microsystems provide a combination of advantages—highly defined flow, reduced diffusion distances, and small size and catalyst requirement—for a variety of applications in sensors, process development, and chemical synthesis. Silicon-based microreactors have the benefit of the mature and refined processes that allow microchannel dimensions of

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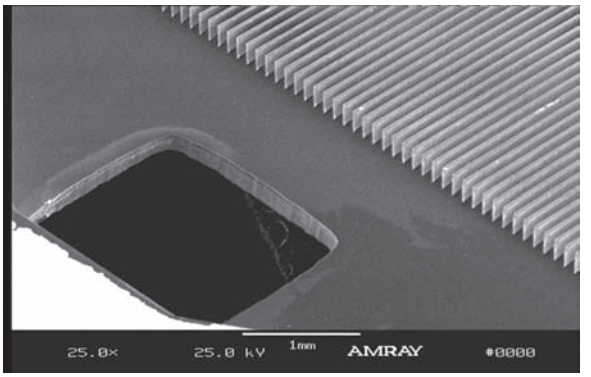

Fig. 1. Scanning electron microscope photograph of inlet and manifold of silicon microreactor.

5 μm or less. Immobilization of enzymes on the surface of silicon *(1)* and silicon microchannels *(2–4)* has been demonstrated in the literature using covalent attachment with various silane linkers. In this work, immobilization of enzymes on silicon walls is performed using layer-by-layer self-assembly.

Layer-by-layer self-assembly is a technique in which thin films are created by sequentially applying oppositely charged polyelectrolytes to a surface *(5)*. In the 1990s, Decher *(6)* proposed this technique and has recently coauthored a book on this subject *(7)*. In 2000, more than 200 articles on polyelectrolyte multilayers were published *(8)*. A screening study demonstrating the deposition of urease on a gold-coated quartz crystal resonator has recently been reported in the literature *(9)*. Polystyrenesulfonate (PSS) and polydiallyldimethylammonium (PDDA) were the polyelectrolytes employed in that study. The present study extends the prior work by applying the multilayers in silicon microchannels, and comparing the efficacy of various architectures through the measurement of first-order rate constants and deactivation rates.

Materials and Methods

Silicon microchannels were produced in a clean-room facility at Louisiana Tech. Photolithography was used to transfer the pattern from a chrome mask to a positive photoresist coated on a <1,0,0> silicon wafer. The channels and vias were etched using an Actel A601 Inductive Coupling Plasma (ICP) employing the Bosch process. The ICP allows high-aspect-ratio vertical sidewalls vs competing technologies such as wet etching. The microreactors consist of an inlet and a manifold, which feed 98 parallel microchannels, each 2.7 cm in length. Each channel is 60 μm wide and 100 μm deep. The channels then feed into a manifold and outlet. Figure 1 is an image from an Amray 1830 Scanning Electron Microscope (SEM) showing the inlet region and microchannels of a microreactor.

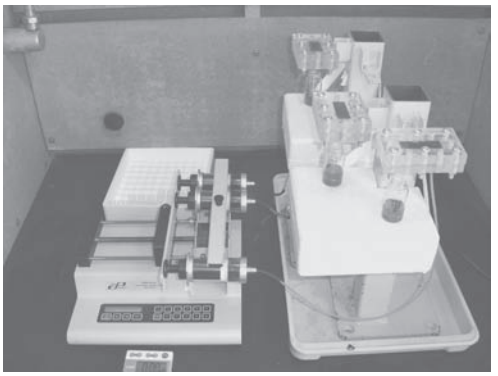

Fig. 2. Experimental setup with multiple reactors.

Urease Canavalia ensiformis with a specific activity of 54,300 U/g was obtained from Sigma (St. Louis, MO). The urea, trizma hydrochloride (Tris-HCl), tris(hydroxy methyl)aminomethane (Tris base), and thymol blue were all ACS reagent-grade products obtained from Sigma-Aldrich. The polycations utilized were polyethylenimine (PEI), polyallylamine (PAH), and PDDA with mol wts of 25,000, 15,000, and 150,000, respectively. The polyanions utilized were PSS and polyvinylsulfate (PVS) with mol wts of 70,000 and 170,000, respectively. Type 1 reagent-grade water was obtained using a Barnstead Series 1090 E-Pure reverse osmosis purifier and utilized for all experiments.

The aqueous feed solution to the microreactor contained 16.7 m*M* urea, 7.6 m*M* Tris-HCl, 8.3 m*M* Tris base, and 42.9 m*M* thymol blue. Thymol blue was chosen owing to its operation in a region optimal for the activity of the urease. The Tris-HCl and Tris base concentrations were chosen to provide sensitivity of the pH indicator over all ranges of urease conversions. A standard curve of the thymol blue indicator was determined using known aliquots of ammonium hydroxide. The end point of 100% conversion was confirmed by testing the absorbance of the feed solution with the free enzyme. The absorbance at 600 nm was measured using an Ocean Optics SD-2000 ultrviolet/visible spectrometer and an AIS mini-DTA deuterium tungsten halogen light source. An extinction coefficient of 27.7 for the production of NH_3 as indicated by thymol blue was determined for this system.

Polyelectrolyte solutions were prepared in aqueous solutions at pH 8.5 and concentrations of 2 g/L for PSS, PEI, and PDDA solutions and 1 g/L for PVS and PAH solutions. Layering was performed by immersing the microreactor into the appropriate polyelectrolyte solution for a period of 10 min followed by a 1-min rinse of Tris buffer. Urease deposition was performed by immersing the microreactor in a 1 g/L solution for 20 min followed by a 1-min Tris buffer wash.

An experimental setup, depicted in Fig. 2, was constructed that allowed three reactors to be operated simultaneously. A syringe pump with three

Table 1
Polyelectrolyte Architectures Tested [a]

Number	Architecture	Day 1 rate constant (s^{-1})
1	PDDA/PSS/PDDA/urease/PDDA	0.0042
2	PDDA/PSS/PDDA/(urease/PDDA)$_4$ [a]	0.0071
3	PAH/PVS/PAH/urease/PAH	0.0040
4	PEI/PVS/PEI/urease/PEI	0.0053
5	PEI/urease/PEI	0.0210
6	PEI/PSS/PEI/urease/PEI	0.0321
7	PEI/PSS/PEI/(urease/PEI)$_6$ [a]	0.1588

[a] Multiple layers of enzyme and polyelectrolytes.

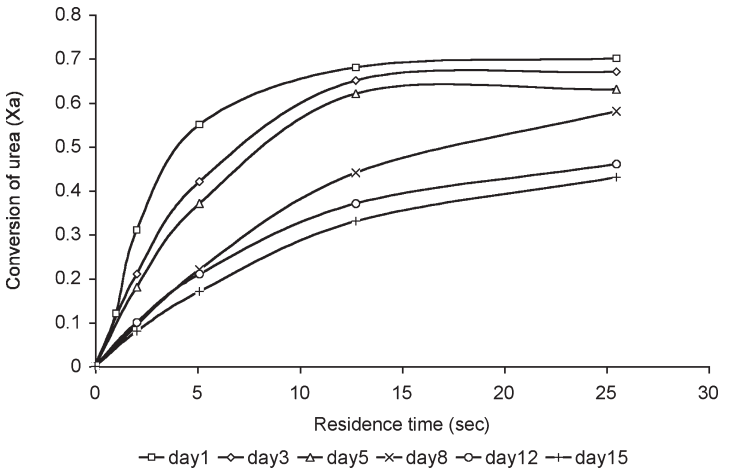

Fig. 3. Conversion rate for PEI/PSS/PEI/(urease/PEI)$_6$.

syringes and feed lines pumped the feed solution into each individual reactor. Samples were collected and analyzed off-line using the Ocean Optics SD-2000 UV/V spectrometer. Different layer-by-layer architectures were applied to each of the reactors to compare the resultant catalytic activity. The experiments were continued over a period of days to assess the decay in activity.

Results and Discussion

Table 1 depicts the architectures tested in our study. As already stated, the enzyme activity of all architectures was characterized by measuring the conversion of urea as a function of microreactor residence time. Figure 3 depicts the activity observed by architecture 7, with each curve representing the activity on a particular day. Figure 4 depicts the first-order rate constant regression using the integral method over the various days of operation. The regressed first-order rate constants were the basis of comparison among enzyme architectures.

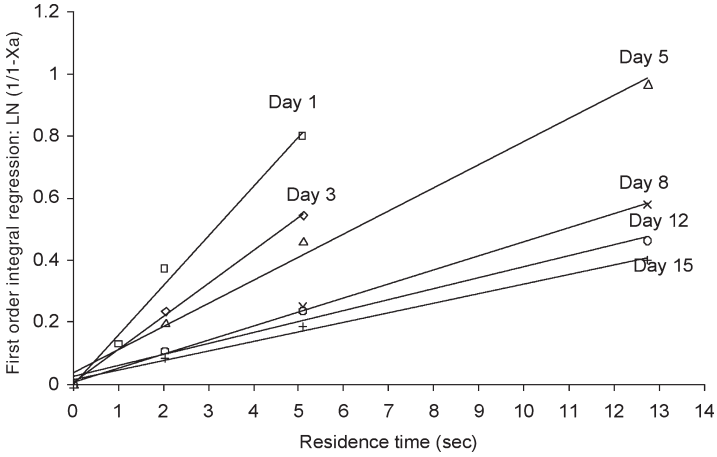

Fig. 4. Rate constant curves for PEI/PSS/PEI/(urease/PEI)$_6$.

Architectures 1 and 2 were based on PDDA as the polycation and PSS as the polyanion. The activity observed for these architectures was very low, even with multiple layers of enzymes, and was abandoned for this reason.

The PEI/PSS polyelectrolytes of architecture 5 were superior to the PAH/PVS and PEI/PVS polyelectrolytes of architectures 3 and 4, respectively, in terms of first-order rate activity. First-order rate activity for d 1 of the PEI/PSS architecture was more than four 4 times greater than that of the PEI/PVS architecture and more than five times greater than that of PAH/PVS architecture (Table 1). Even on d 20 of operation, the first-order rate activity of the PEI/PSS reactor was higher than for the other two architectures on the first day of operation. The PEI/PSS reactor produced conversions of about 60% at 102 s of residence time on d 1, compared with 17 and 24% for the PAH/PVS and PEI/PVS reactors, respectively. By d 20, the PEI/PSS reactor produced a peak conversion of 18% at the highest residence time tested. Peak conversion rates for the PAH/PVS and PEI/PVS reactors had dropped to 5 and 10%, respectively. For this reason, further efforts concentrated on PEI/PSS-based architectures.

An experiment was performed making a PEI/urease architecture without the PEI/PSS precursor layer (architecture 5). The PEI/PSS precursor layer (architecture 6) was found to have a 50% higher d 1 first-order rate constant. A 26-s residence time resulted in 50% conversion with the precursor layer and only 25% conversion without the precursor layer.

An enzyme architecture with the PEI/PSS precursor layer and six multiple layers of PEI/urease was compared with a similar architecture containing only one enzyme layer. As expected, the increase in enzyme layers dramatically increased the activity of the reactor. The six layers of enzyme had a first-order rate constant five times higher than the single-

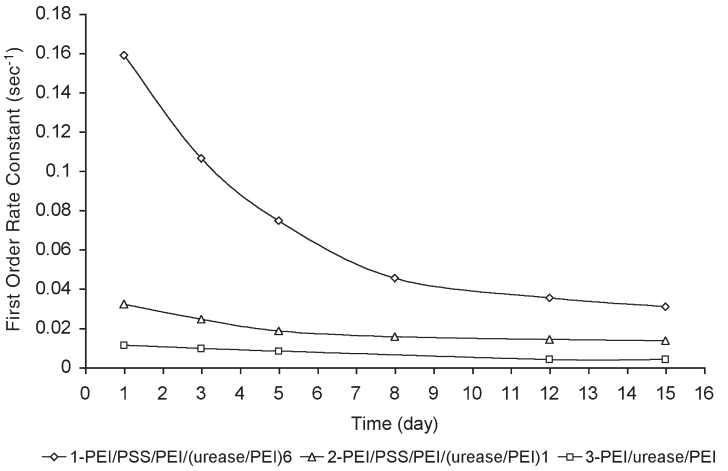

Fig. 5. Comparison of first-order rate decay.

layer experiment (Table 1). The conversion of the single-layer and six-layer enzyme architecture was 50and 70%, respectively, on d 1 for a residence time of 26 s. Figure 5 depicts the decay rate of the first-order rate constants for the three PEI/urease architectures tested. The six-layer architecture exhibited an exponential decay in activity. The single-layer architectures had much lower initial activities but retained this activity at a relatively constant rate, especially after the initial decay observed in the first 5 d of operation. Reactor product samples once obtained were monitored successively over time (i.e., a period of days) and no further reaction was noted. This evidence suggests that the decay in activity was not owing to losses of the enzyme into the product effluent but, rather, to denaturing of the enzyme. Protein assays of the reactor and effluent are under way and will be reported at a later date.

Conclusion

Layer-by-layer nano self-assembly is a convenient and inexpensive technique for immobilizing enzymes on the surface of silicon microreactors. Extremely high surface area to volume available in a microreactor provides a maximum opportunity for reactions at the channel walls. Of the architectures tested, PEI is the best choice for immobilizing urease. A precursor layer of PEI/PSS was found to increase significantly the activity of the subsequent enzyme layers. Microreactors with layer-by-layer encased enzymes showed significant activity after more than 2 wk. The conversion rate for the best microreactor in our study exhibited 70% conversion on d 1 of testing and was still converting >50% of urea solution at residence times under 1 min after 15 d. Experiments using microreactors with channel widths of 5 μm are ongoing. The reduced diffusion distances should

further demonstrate the advantage of the microreactor compared with other reactors with similar surface areas.

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