A critical requirement for achieving a micro total analytical system for the analysis of cells and their constituent proteins is to integrate the lysis and fractionation steps on-chip. Here, an experimental microfluidic system integrating the lysis of bacterial cells and the extraction of a large intracellular enzyme, β-galactosidase, is demonstrated. The β-galactosidase is detected and quantified using a fluorogenic enzyme assay and a numerical model. While the focus is on the lysis of typical Gram-negative bacterial cells (E. coli), the techniques described here could, in principle, be applied to a variety of different cell types.

The field of microfluidics for chemical, biochemical, and cellular analysis on small integrated microchips is quickly demonstrating its potential for providing invaluable clinical and research tools. Among others, some interesting technologies available in a microfluidic format include capillary electrophoresis,1 isoelectric focusing,2 immunoassays,3,4 sample injection of proteins or analysis via mass spectrometry,5,6 PCR amplification,7,8 and DNA analysis.9,10 However, many of these techniques currently require off-chip lysis of cells. Typical laboratory protocols for these off-chip lysis steps include the use of enzymes (lysozyme), chemical lytic agents (detergents), and mechanical forces (sonication, bead milling). However, many such lysis techniques are not amenable to implementation in a microfluidic format. The ability to integrate the lysis of cells with the analysis of their contents would greatly increase the power and portability of many microfluidic devices.

Several other research groups have developed microfluidic cell lysis devices. For example, an integrated monolithic microchip device was fabricated that used electrokinetic fluid actuation and thermal cycling to accomplish lysis of Escherichia coli and PCR amplification of DNA.11 In a similar electrokinetic device, the controlled manipulation of canine erythrocytes throughout a channel network and dark-field images of SDS lysis of the cells at a T-junction were demonstrated.12 Several groups have reported the use of minisonicator devices in conjunction with microfluidics for the lysis of bacterial spores.13,14 In a different approach, a silicon channel was fabricated with multielectrode pairs along the walls to deliver an electric field to irreversibly electroporate several different cell types.15 A voltage of 10 V was applied across gaps of several micrometers to achieve electric fields on the order of 1 to 10 kV/cm.

These approaches have been shown to be moderately successful. However, they all depend on the use of an external power supply and the devices may be quite complicated and costly to fabricate. Little work has been published with regard to cell lysis in pressure-driven microfluidic channels.

Previous work from this laboratory developed two simple microfluidic devices that exploit transverse diffusion across two or more parallel laminar flow streams.16 The H-filter and the T-sensor were developed for sample extraction and chemical detection, respectively. Typically, these microfluidic devices have been demonstrated in a manner in which they operate independently and carry out one chemical or physical process at a time. Demonstrated here is a relatively simple diffusion-based microfluidic system that allows the continuous lysis of bacterial cells as well as the fractionation and detection of specific intracellular components. This is the first report demonstrating a complex set of preparative and detection processes carried out in a series of two such devices in tandem. The first device is an H-filter that performs two functions “at once”—the lysis of the cells, followed by the fractionation of proteins. The second device is a T-sensor for chemical analysis.

**Cell Lysis and Protein Extraction in a Microfluidic Device with Detection by a Fluorogenic Enzyme Assay**

Eric A. Schilling, Andrew Evan Kamholz, and Paul Yager*

Department of Bioengineering, University of Washington, Box 352255, Seattle, Washington 98195

*To whom correspondence is to be addressed. E-mail: yagerp@u.washington.edu.


1798 \*Analytical Chemistry, Vol. 74, No. 8, April 15, 2002 10.1021/ac015640e CCC: $22.00 © 2002 American Chemical Society Published on Web 03/08/2002
in the same channel by extraction of medium- to low-molecular-weight solutes released from those cells. The second device is one in which an enzymatic hydrolysis step operates concurrent with optical detection of the progress of the upstream processes, both in a single T-sensor. Critical to successful integration of the system was the development of an accurate model of the mass transport and enzymatic reactions in the devices.

**Device Design.** The device described here consists of three inlets, two outlets, and two central channels (Figure 1). On the basis of their function, the main channels are named the lysis channel and the detection channel. To achieve predictable flow patterns, pump rates are controlled at all inlets and one outlet. Lytic agent diffuses into the cell suspension, lysing the cells. Intracellular components then diffuse away from the cell stream and some are brought around the corner into the detection channel, where their presence can be detected by the production of a fluorescent species from a fluorogenic substrate.

Figure 1. Schematic of microfluidic device for cell lysis and fractionation/detection of intracellular components. Pump rates are controlled at all inlets and one outlet. Lytic agent diffuses into the cell suspension, lysing the cells. Intracellular components then diffuse away from the cell stream and some are brought around the corner into the detection channel, where their presence can be detected by the production of a fluorescent species from a fluorogenic substrate.

**Experimental Section**

**Integrated Lysis and Protein Fractionation/Detection Device.** A three-inlet, two-outlet microfluidic device was designed for the continuous lysis of bacterial cells and the fractionation/detection of intracellular proteins (Figure 1). The main channel width was 1000 μm, the depth was 100 μm, and the length was 4 cm. A suspension of E. coli cells (~10^9 cells/mL) and 1 mL/mL Bacterial Protein Extraction Reagent (BPER; Pierce, Rockford, IL), a proprietary mild detergent designed to allow the release of large proteins, were pumped at 10 nL/s through separate inlets into the lysis channel. These flow rates were chosen on the basis of the CoventorWare numerical model shown below of the diffusion of β-gal in a similar geometry under pressure-driven flow conditions. The flow rate at the controlled outlet was set to ~14 nL/s such that the entire cell stream was pumped to waste. The removal of cells was verified by imaging cells under dark-field illumination with background subtraction and by fluorescence staining. A 200 mM solution of resorufin β-d-galactopyranoside (RBG) was pumped in the third inlet at a flow rate of 10 nL/s. The average residence time in the lysis channel was 190 s, and due to the asymmetry in flow rates, that in the detection channel was 250 s.

**Device Fabrication.** Microfluidic devices were fabricated using a CO2 laser cutting tool (Universal Laser Systems, Inc., Scottsdale, AZ) at the Washington Technology Center (Seattle, WA). The laser cutting tool attached to a PC was used to cut the appropriate device outlines in Mylar laminates (Fraylock, Inc., San Carlos, CA). The protective layers over the adhesive were removed and the laminates were assembled into a composite device. Due to the ablation of Mylar during laser cutting, a finite surface roughness has not been studied in great detail but likely does allow small bubbles to be trapped along the walls and for increased molecular adsorption. However, because the assay described here depends only on the behavior of particles along the centerline of the channels, surface roughness does not have a major effect on the results.

**Fluidics and Optical Detection.** The device was mounted in a custom-made polycarbonate/aluminum manifold. Polyetheretherketone (PEEK; Upchurch Scientific, Oak Harbor, WA) tubing was used to supply fluids to the device from PC-controlled 50 μL syringe pumps (Kloehn Co. Ltd., Las Vegas, NV). Images were captured with a frame-grabber card (CG-7; Scion Corp., Frederick, MD), a three-chip cooled CCD camera (ChromoCam 300, Oncor, Gaithersburg, MD), and an inverted epifluorescence microscope (IM 35 inverted microscope; Zeiss, Thornwood, NY). A 10×...
objective was used with a 100-W mercury arc source (Zeiss) providing excitation light. The intensity of excitation light delivered to the sample was measured using a power meter (model 212, Coherent Inc., Auburn, CA) and found to be ~40 W/ cm² through a fluorescein filter set.

An incandescent light was used to illuminate the microfluidic channel to obtain dark-field images. To achieve good dark-field signal intensities, E. coli cells grown overnight were resuspended in 500 µL of buffer, imaging was conducted using a 2.5× objective, and the incandescent lamp was adjusted to a very oblique angle, illuminating along the length of the channel. A background image (channel containing only water) was subtracted from sample images to produce images in which cells appeared as dark spots on a white background. Different detergents used to lyse E. coli cells were found to have different effects on dark-field images. Under either static or flow conditions in a T-sensor, 100 mM sodium dodecyl sulfate (SDS) was found to cause a significant decrease in scattered signal, indicating complete degradation of the membrane, the cytosol, or both. However, using BPER, very little change in refractive index of the cell suspension was observed even after 18 min. These results, along with others using fluorescence (data not shown), indicate that while 100 mM SDS causes the complete disruption of cells, BPER seems only to increases the permeability of the membrane. The increase in permeability due to BPER is expected to allow large molecules such as β-gal to exit cells. Although SDS was used in some preliminary portions of this study, it is not an ideal choice of detergents for the complete assay as it is generally quite damaging to proteins. A simple fluorometer study demonstrated that BPER did not effect the catalytic activity of β-gal, and because BPER was designed specifically for the extraction of intact proteins, the effect of this detergent on most molecules should be mild.

**3D Numerical Modeling.** To better understand the feasibility of a microfluidic device for the lysis of bacterial cells and the fractionation of diffused intracellular components, three-dimensional simulations were performed using CoventorWare (Covington, Cary, NC). As with the experimental device, the modeled device had channels that were 1000 µm wide and 100 µm deep, and the length of each main channel was 4 cm. Flow rates of 10 nL/s were specified at the three inlets and a flow rate of ~10 nL/s was specified at the controlled outlet. These flow rates are equivalent to 10 steps/s using the experimental Kloehn syringe pumps described above. After the velocity profile throughout the device was calculated, a species concentration boundary condition was specified at the cells inlet, producing the distribution of analyte throughout the device (Figure 2A). In this case, the analyte was β-gal, with a diffusion coefficient of 2.7 × 10⁻⁸ cm²/s, based on literature values.

The predicted concentration profiles across several locations in the device are plotted in Figure 2B and demonstrate that a substantial fraction of β-gal can be separated from the cell stream.

It is important to note that the simulation assumes that β-gal is immediately able to begin diffusing at the inlet of the device. In an actual device, the lytic agent must first diffuse into the bacterial cell stream and the membranes must be significantly permeabilized. Only then is the intracellular β-gal free to begin diffusing. Based on previous lysis experiments (data not shown), the time required for the diffusion of lytic agent and the disruption of E. coli membranes is on the order of several seconds. At pump rates of 10 nL/s, the average residence time in the lysis channel is 190 s. This allows β-gal to diffuse an average distance of nearly 100 µm. Because the lysis time is exceeded by the total residence time by as much as 2 orders of magnitude, the error caused by this assumption should be negligible.

**Detection of Intracellular Components.** A fluorogenic enzyme assay was used to detect extracted intracellular components. RBG is a fluorogenic substrate for β-galactosidase. β-gal catalyzes the reaction from RBG to resorufin and β-galactose. β-gal is naturally expressed in E. coli at low levels (~60 molecules/cell) but its expression can be constitutively induced by isopropyl β-D-thiogalactoside (IPTG). When excited at 570 nm, resorufin fluoresces brightly at 594 nm, with a quantum efficiency over 1000 times greater than that of RBG excited at the same wavelength. On the basis of the design shown in Figure 1, RBG will only be cleaved to produce resorufin when the intracellular β-gal has been released from the cells and has been diffusively separated from the cell stream. This particular fluorogenic substrate assay has been used successfully in the past, both for single-cell analysis

---

1800 Analytical Chemistry, Vol. 74, No. 8, April 15, 2002

---

and in microfluidic systems. Experimental measurements of the kinetic parameters for this reaction in a microchannel are shown below.

Cell Culture. In all cases, bacterial cells were cultured using standard microbiology techniques. E. coli (ATCC HfrH-) stocks were stored in a −80 °C freezer until use. This stock was used to inoculate 5 mL of a rich growth medium, 1% (w/v) tryptone, 1% (w/v) NaCl, and 0.5% (w/v) yeast extract containing 520 μM IPTG. The culture tubes were placed in a stirring water bath at 37 °C and 150 rpm overnight.

Using measurements of the optical density and the extinction coefficient of typical cell suspensions, Beer’s law was used to calculate that a typical experiment involving cells grown overnight had a density of ~4 × 10^8 colony forming units/mL.

Numerical Model. A new version of a previously described one-dimensional analytical model was used to fit experimental results and determine the enzyme concentration. The finite difference model, based on a two-stream flow within a T-sensor, includes parameters such as channel dimensions, flow rate, diffusion coefficients, and enzyme reaction kinetics. The model was implemented in MATLAB and has been shown to accurately predict diffusion coefficients, protein concentrations, and kinetic parameters. The improvement to the model presented for the first time here is the incorporation of enzymatic conversion of substrate to product.

Development of the present model started with the traditional Henri–Michaelis–Menten relationship for enzymatic reactions:

\[ v = \frac{V_{\text{max}} [S]}{K_m + [S]} \]

where \( v \) is the instantaneous reaction velocity, \( V_{\text{max}} \) is the maximal reaction velocity for the given chemical environment, \( S \) is the substrate, and \( K_m \) is the Michaelis constant. In the case of the T-sensor assay, the enzyme concentration varies over position and therefore \( V_{\text{max}} \) is not constant. However, the local \( V_{\text{max}} \) can be expressed as the product of \( k_p \), the rate constant for conversion of the enzyme–substrate complex to product, and the total local enzyme concentration:

\[ V_{\text{max}} = k_p [E] \]

The combination of eqs 1 and 2 leads to a universal relationship for which the instantaneous reaction velocity can be calculated from the local enzyme and substrate concentrations:

\[ v = \frac{k_p [E][S]}{K_m + [S]} \]

Equation 3 assumes that the reverse reaction (product conversion to substrate) is negligible.


The present one-dimensional model considers the diffusion of substrate, enzyme, and product, as well as the catalysis of substrate to product. The system of equations was based on a similar system in an earlier publication, with the reaction terms changed to enzymatic expressions:

\[ \frac{∂[S]}{∂t} = D_s \frac{∂^2 [S]}{∂x^2} - \frac{k_p [E][S]}{K_m + [S]} \]  
\[ \frac{∂[E]}{∂t} = D_E \frac{∂^2 [E]}{∂x^2} \]  
\[ \frac{∂[P]}{∂t} = D_p \frac{∂^2 [P]}{∂x^2} + \frac{k_p [E][S]}{K_m + [S]} \]

where \( D_i \) is the diffusion coefficient of species \( i \) and the coordinate axes are as in Figure 3. In the present study, \( S \) is RBG, \( E \) is β-gal, and \( P \) is resorufin. Note that utilization of Henri–Michaelis–Menten kinetics eliminates the need to consider the concentration of enzyme–substrate complex.

To validate the model for the RBG–β-gal system, a simplified set of T-sensor experiments was conducted. In this scenario, the enzyme was introduced on one side of the main channel while the RBG was introduced on the other side (Figure 3). As the two analytes interdiffused, catalysis proceeded, producing resorufin. These experiments used a much simpler T-sensor than the one used for the main experiments in the present study. The validating experiments were done in a silicon and glass device with a main channel that was 1800 μm across and 10 μm deep. This device, including fabrication, utilization, and detailed quantitative analysis, has been described in great detail elsewhere.

For diffusion coefficient values, the model used \( D_{\text{resorufin}} \) of 4.8 × 10^{-6} cm^2/s and \( D_{\text{RBG}} \) of 4.3 × 10^{-6} cm^2/s, as determined by simple T-sensor diffusion coefficient measurements using the identical protocol discussed elsewhere. For β-galactosidase, a diffusion coefficient of 2.7 × 10^{-7} cm^2/s was used. The \( K_m \) value was determined by the traditional Lineweaver–Burke method, using fluorometric determination of product generation. The measured value of 538 ± 63 μM compares quite favorably with a published value of 500 ± 200 μM.

The experimental setup is shown in Figure 3. In separate trials, RBG concentrations of 255.7 and 25.6 μM were introduced on the right side of the T-sensor. The β-gal was introduced on the left side at 0.25 mg/mL, corresponding to a concentration of ~0.463 μM. Measurements were taken 5000 s downstream from the inlet. The total flow rate was 5.21 nL/s.

When these experimental conditions were modeled, the only unknown parameter was \( k_p \). The best fit to the two data sets, shown in Figure 3, yielded a \( k_p \) value of 70 1/s, which is identical to a previously published value of 70 ± 30 1/s. This result validates the model as an accurate tool for describing the enzyme-based assay in the T-sensor. In the rest of this study, this model uses the same parameters used in the validating experiments to characterize performance of the lysis device.

RESULTS AND DISCUSSION
Characterization of Flow Properties. Before proceeding to the complete assay in a Mylar device, the flow properties within the device were characterized to ensure stability and reproduc-
A 100 μM solution of resorufin in 100 mM MES buffer was pumped into the cells inlet while buffer was pumped into the other inlets of the device at flow rates of 40, 200, and 400 nL/s (Figure 4A-C, respectively). Although some bubbles were present along the channel walls due to poor priming of the device, the general flow pattern was as expected. Most of the resorufin was carried out the controlled outlet at the left of the images. The resorufin that had diffused into the right half of the lysis channel was split off at the junction and carried around into the detection channel. Because resorufin is a quickly diffusing molecule, the amount that was extracted at the junction depended heavily on flow rate. Based on the average residence time, the calculated average diffusion distance of resorufin in the lysis channel at inlet flow rates of 40, 200, and 400 nL/s was 227, 101, and 72 μm, respectively.

Assay Results. After verifying that the flow properties within the device were as expected, the full lysis and detection assay was performed. A suspension of E. coli cells was pumped into the cells inlet while BPER was pumped into the lytic agent inlet. A 200 μM solution of RBG was pumped into the detection molecule inlet. These inlet flow rates were controlled at 10 nL/s. The controlled outlet flow rate was held at 14 nL/s to ensure complete removal of cell bodies and other very large molecular weight components. Using the appropriate filter set (BP 570, LP 600), fluorescence images where captured at various locations in the device (Figure 5A). The production of resorufin was observed at the expected location, and the fluorescence intensity increased as flow progressed downstream due to the continuous enzymatic catalysis. As a control, when the lytic agent was replaced with distilled (DI) water, β-galactosidase was not able to exit the cells, and consequently, no resorufin was produced in the detection channel (image not shown).

To more rigorously verify that no cells or cell bodies entered the detection channel, dark-field images of the device were captured just before the fluorescence images (Figure 5B). In a typical dark-field image, cells appear as light spots on a dark background. However, in the image discussed here, a background image was subtracted from the experimental image to remove scattered signal due to imperfections in the Mylar. This resulted in an image in which cells appear as black spots on a light background. Capturing fluorescence and dark-field images within a few seconds of one another, it can be seen that no cells were transported into the detection channel while a strong resorufin signal was produced (Figure 5A,B). In a separate method to verify that no cells entered the detection channel, a DNA intercalating dye, SYTO 9, was used along with a dual band-pass filter set to visualize both the DNA and the production of resorufin at the same flow rates (Figure 5C). In this case, it can be seen that the SYTO 9-labeled DNA (green) is removed from the device while the enzymatic production of resorufin (red) still occurs in the detection channel. This indicates that some β-gal was extracted from the cell stream and that no cells reached the detection channel. The fact that a higher flow rate at the controlled outlet (14 vs 10 nL/s) was required to evenly split the two streams suggests that the cell suspension was more viscous than the lytic agent stream, which is consistent with the presence of DNA in solution.

Measurement of Resorufin Diffusion Coefficient. To allow accurate comparison between experimental and model results, the effective diffusion coefficient of resorufin in the device was measured. This was accomplished by collecting diffusion profiles of resorufin in the detection channel. Using the 1D model to find the best fit with these diffusion curves, the diffusion coefficient of resorufin in buffer was found to be $6.6 \times 10^{-6}$ cm$^2$/s. This value of the diffusion coefficient of resorufin is 37% higher than the value measured previously in a smaller silicon device.

There are several credible explanations for this difference in diffusion coefficient for resorufin. First, previous measurements
were made in a device with curved inlet legs and measurements were made very far downstream, minimizing the effects of entry length and the stagnation point. Because the device used here does not have curved inlet legs, diffusion coefficient measurements are likely to be overpredicted due to the increased residence time at the inlet. Second, there may be some uncertainty in flow rate as an input parameter to the 1D model due to bulging of the Mylar under flow conditions. In addition, as discussed in the literature, effective diffusion coefficient values can vary from expected values when the depth of the device is substantially larger than 10 μm, as in the present study (depth, 100 μm). Such effects are due to the influence of the velocity profile and secondary concentration gradients that develop through dispersion. However, because the resorufin diffusion coefficient was measured in the same device at the same flow rate in which the enzyme assay was carried out, it can be treated as the effective diffusion coefficient for the diffusion of resorufin regardless of the true diffusion coefficient and can be used accurately for fitting model data.

**Quantification of Enzyme Concentration.** This assay and similar enzyme-based assays can be made quantitative by fitting experimental results with predictions from a 1D numerical model. First, intensity profiles across the width of the channel at various time points are collected. Based on the initial concentration of fluorogenic substrate, known reaction parameters (K_m, k_cat) and Henri–Michaelis–Menten kinetics, the initial model enzyme concentration can be varied until numerical results match experimental results. However, a number of assumptions inherent in the model must be considered. First, the model assumes that the flow development region and other three-dimensional effects are negligible. This assumption is generally valid for measurements made a long distance (or a long residence time) from the inlet. Second, the model assumes that diffusion coefficients and reaction kinetics are unaffected by the presence of detergent. While BPER is likely to affect reaction kinetics to some extent, the effects have been shown to be relatively small. However, it is not clear what effect BPER had on the diffusion of molecules within the device.

An important consideration for the model is that the enzyme inlet boundary condition must be specified to reflect the fact that β-gal is not uniform (is not a step function) across the channel as it reaches the detection channel. Several numerical simulations...
were performed to explore the effects of using a uniform versus a nonuniform inlet condition for \( \beta \)-gal on the resulting fluorescence intensity profiles. Input parameters were based on experimentally measured values (\( K_m = 538 \mu M \), \( k_{cat} = 70 \text{ 1/s} \), \( D_{resorufin} = 6.6 \times 10^{-6} \text{ cm}^2/\text{s} \), \( D_{RBG} = 4.3 \times 10^{-6} \text{ cm}^2/\text{s} \)) or set based on literature values (\( D_{enzyme} = 2.7 \times 10^{-7} \text{ cm}^2/\text{s} \)). The total flow rate was 20 \( \text{ nL/s} \), the same as that for most experimental conditions. Using an initial RBG concentration of 50 \( \mu M \) and the nonuniform \( \beta \)-gal profile predicted by the 3D CoventorWare model (see Figure 2) with a maximum concentration of 120 nM, the effect of using a uniform versus a nonuniform concentration profile was quite apparent (Figure 6A). This demonstrates that the inlet concentration profile of \( \beta \)-gal has a significant effect on the predicted fluorescence within the channel and the more realistic case, a nonuniform profile, should be used for quantitative comparisons with experimental data.

Using the measured effective diffusion coefficient of resorufin (\( D = 6.6 \times 10^{-6} \text{ cm}^2/\text{s} \)), transforming the fluorescence intensity values to resorufin concentration based on an experimentally measured curve, and using the nonuniform inlet \( \beta \)-gal concentration predicted by the CoventorWare model, the 1D model was used to fit experimental results at two downstream locations (Figure 6B). By varying only the enzyme concentration, the best fit to experimental data was achieved when the maximal \( \beta \)-gal concentration was 165 nM. Therefore, the maximal concentration of \( \beta \)-gal extracted from the cell stream under these conditions was \( \approx 165 \text{ nM} \). Based on the simplified model predictions shown in Figure 2, \( \approx 10\% \) of the \( \beta \)-gal present in the cell stream was fractionated and delivered to the detection channel.

**CONCLUSIONS**

A microfluidic system integrating the continuous lysis of bacterial cells and the fractionation/detection of a large intracellular protein has been demonstrated. Through the use of dark-field illumination, fluorescence, and a fluorogenic enzyme assay, the fractionation of cells from an intracellular enzyme, \( \beta \)-gal, has been demonstrated. A technique for the quantification of the concentration of a particular intracellular component was demonstrated. This assay serves as a proof-of-principle assay for future cell lysis and intracellular product detection devices and demonstrates the feasibility of a continuous-flow, pressure-driven microfluidic device for the lysis of cells and fractionation of intracellular components.

Because \( \beta \)-galactosidase is a large molecule, the ability to successfully fractionate these molecules from larger molecular weight components (such as intact cells and DNA) suggests that the assay is equally feasible for fractionating smaller molecules from the cell stream by further adjusting the controlled outlet flow rate. For example, by increasing the controlled outlet flow rate, few large molecules will be able to diffuse into the detection channel, thereby increasing the purity of small molecules. However, the purpose of this device is to roughly fractionate molecules based on their size and to allow estimates of the concentration of a particular fractionated molecule rather than to provide a tool for high-efficiency separation. Consequently, it will be necessary to integrate this device with other downstream microfluidic devices to provide a complete analytical tool. The integration of these components should be relatively straightforward using similar Mylar fabrication techniques or other microfabrication techniques and could include capillary electrophoresis or isoelectric focusing. Another logical extension of the work would be to place a microfluidic mixer upstream of the detection channel. This would allow more complete lysis of the cell suspension and liberate more intracellular enzyme before the diffusive separation of enzyme and cells.

**ACKNOWLEDGMENT**

This work was supported by the Department of Bioengineering, the Washington Technology Center, and Micronics, Inc. The authors thank members of the Yager research group, including Mr. Anson Hatch, M.S. Catherine Cabrera, Mr. Kenneth Hawkins, and Mr. Hugh Chang for helpful scientific discussions and especially to Mr. Matthew Munson for assistance in running some numerical model simulations.

Received for review October 1, 2001. Accepted January 29, 2002.

AC015640E