MONITORING AND CONTROL OF CELL GROWTH IN FED-BATCH MICROBIOREACTORS

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ABSTRACT
We present a fed-batch microbioreactor made of poly(methylmethacrylate) (PMMA). The reactor chamber has a working volume of approximately 80μℓ and includes an active magnetic mixer to maintain uniform concentration distribution. The reactor accommodates fluorescent sensors for on-line measurement of pH and dissolved oxygen (DO), as well as transmission measurement of cell density. *Escherichia coli* cell growth has been demonstrated for both batch and fed-batch processes.

Keywords: Evaporation-driven flow, measuring dissolved oxygen, microbioreactor, pH monitoring

INTRODUCTION
The driving force behind research in microbioreactors with integrated sensors and actuators is the demand for high-throughput screening platforms for bacterial strains and bioprocess operations. Previously, we reported on 5μℓ batch microbioreactors machined of poly(dimethylsiloxane) (PDMS) [1]. Compared with batch processes, fed-batch operations allow for controlling of nutrients concentration or pH and are thus more widely used in bioprocess development [2]. We have realized a fed-batch microbioreactor using an evaporation-driven passive pumping system. Different from the previous batch microbioreactor design, the structural parts of the fed-batch reactor are machined in PMMA, which yields greater mechanical rigidity and lends itself to plastic microfabrication methods. Additionally, the larger working volume of approximately 80μℓ provides more cell material for subsequent gene expression analysis.

DESIGN OF FED-BATCH MICROBIOREACTOR
The fed-batch microbioreactor consists of a round chamber (diameter 10mm, depth 1mm) and three connecting channels (depth 500μm, width 500μm), which are used for inoculation and reagent-feeding (Figure 1). A thin layer of spin-coated PDMS covers the chamber and serves as aeration membrane. This thin PDMS layer is held by a thicker PDMS layer to facilitate device assembly, sealing and microfluidic connections. Two recesses (diameter 2mm, depth 250μm) at the bottom of the bioreactor chamber accommodate pH and DO fluorescence lifetime sensors. A 6mm long magnetic spin bar in the chamber center mixes the fermentation medium. The spin bar rotates around a vertical post machined out of the bulk PMMA.

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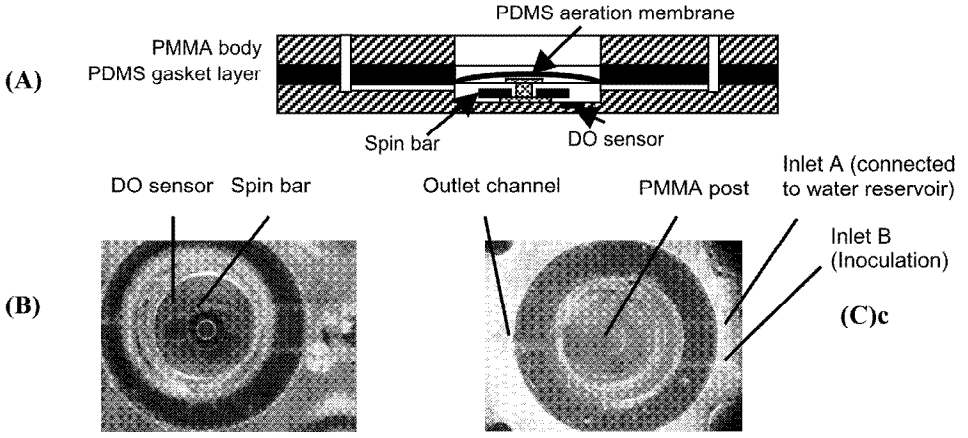

Figure 1: A – Schematic longitudinal section of the microbioreactor; B – Photograph of the empty chamber of the reactor. The spin bar in the center and the fluorescent sensor for DO (black spot) are visible; C – Photograph of the chamber at the end of the run. Turbidity of the cell culture obscures the spin bar and the DO sensor.

EXPERIMENTAL SET-UP AND EVAPORATION-DRIVEN FEEDING

Fermentations are carried out in an incubator chamber kept at 37°C by flowing heated water through its base. One inlet channel connects the microbioreactor to an elevated water reservoir. This pressure passively pumps liquid at the same rate as water evaporates through the thin PDMS layer, thus keeping the volume of the microbioreactor constant. In principle, the pumping rate can be adjusted by controlling the humidity in the incubator. The cell culture is operated as a batch process when water is fed into the microbioreactor, or as a fed-batch process when other solutions (e.g. glucose or base) are drawn into the microbioreactor by water evaporation (~μℓ/hr).

The incubator chamber is placed directly above a magnetic stirrer to minimize the distance to the spin bar in the microbioreactor (Figure 2). Bifurcated optical fibers lead into the chamber from both the top and the bottom and are each connected to different LEDs and photodetectors. A transmission measurement using an orange LED (Epitex L600-10V, 600nm) returns the optical density. The DO and pH sensor patches are excited with a blue-green LED (Nichia NSPE590S, 505nm) and a blue LED (Nichia NSPB500S, 465nm) respectively. Exciter bandpass filter (Omega Optical XF1016 and XF1014) and emission longpass filters (Omega Optical XF 3016 and XF 3018) separate the respective excitation and emission signals and minimize cross-excitation. Data switches multiplex the output signal and the input signal of the function generator and the lock-in amplifier, respectively. All instruments are PC-controlled under a LabView software routine, which allows for automated and on-line measurement of the parameters. For the results shown below, the three parameters were read every 10 minutes.

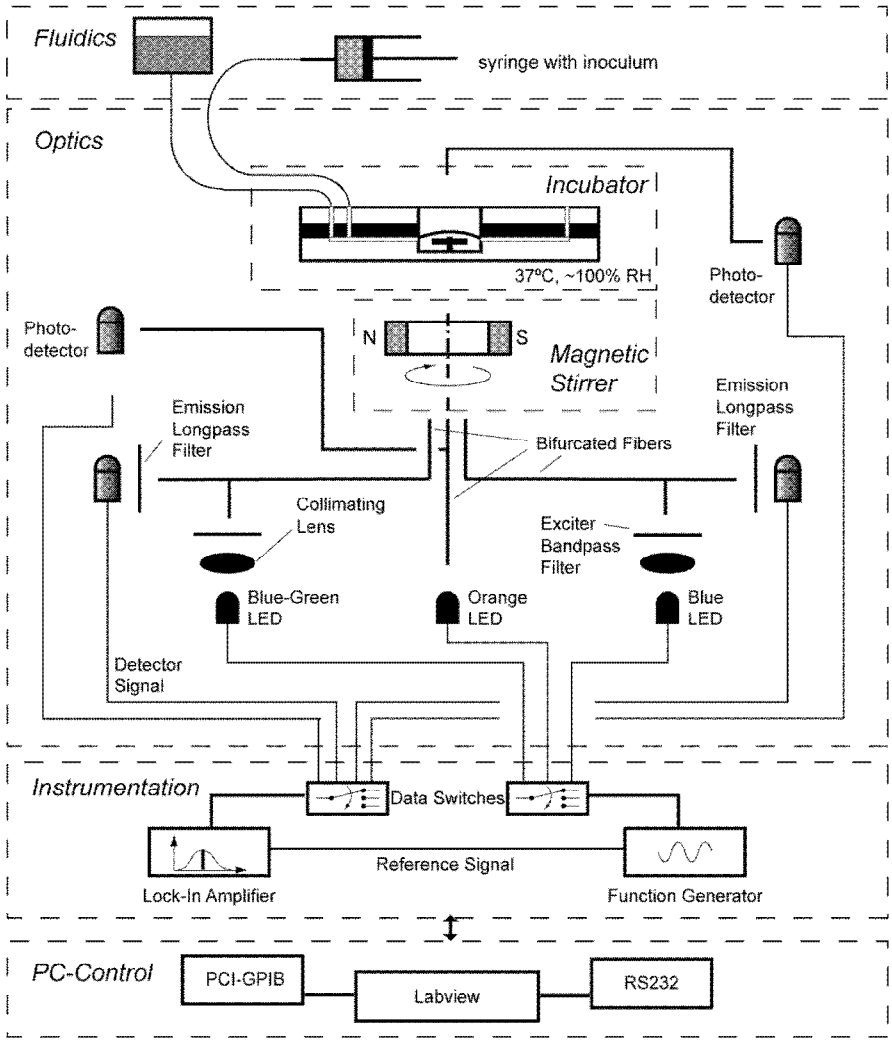

Figure 2: Schematic of the experimental set-up with the instrumentation, the optics, the magnetic stirring, the incubator chamber and the fluidics for reagent feed and inoculation (syringe not attached during run).

RESULTS

Figure 3 shows DO and pH curves for typical *E. coli* fermentations in the microbioreactor. For batch fermentation, the DO level drops rapidly to zero during exponential growth phase, when the multiplying cells have a strong demand for oxygen. As the cells enter the

stationary phase, the oxygen demand drops and diffusion across the PDMS membrane returns the DO level to saturation. Addition of nutrient (glucose) appears to increase the length of the growth phase, slowing the return of the DO to saturation level.

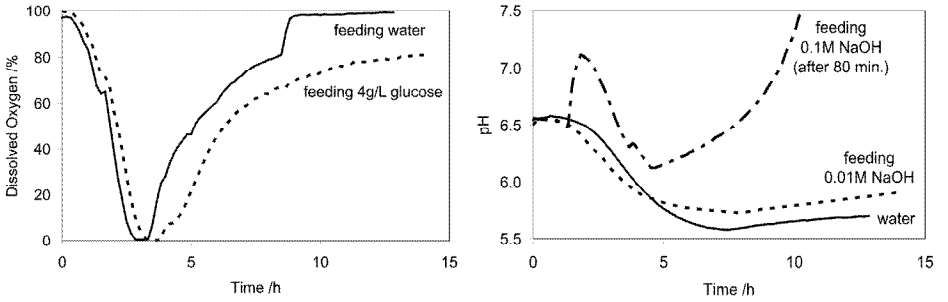

Figure 3: (left) DO for one batch and one fed-batch fermentation run with glucose in the microbioreactor; (right) pH for one batch and two fermentation runs feeding base solution. For the 0.1M NaOH solution, the feeding started at 80 min.

The pH curves show a decrease to pH 5.6 in batch fermentation, which is reduced when a diluted base solution (0.01M NaOH) is fed. When a strong base solution (0.1M NaOH) is fed, pH decreases even less during cell growth phase and strongly increases thereafter. In the shown example, the strong base solution was administered 80 minutes after the fermentation run had started with cell growth in early phase.

CONCLUSIONS

The results demonstrate that the environmental conditions in microbioreactors can be monitored as in a batch process and manipulated in a fed-batch process. Moreover, if pH values are maintained close to neutral, cell density is expected to increase. Thus, the ability to feed base and nutrients makes this micro fed-batch system a promising method for screening applications in bioprocess engineering.

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